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OFFICE OF PETITIONS

In re Patent No. 6,030,790 :
Issue Date: February 29, 2000 :
Application No. 08/817,547 : ON PETITION
Filed: March 27, 1997 :
Patentee: Adermann et al. :

This is a decision on the petition and papers filed by a third party under 37 CFR § 1.182 or 1.183 on July 15, 16, 19, 2002, which collectively oppose favorable treatment of the request for a Certificate of Correction asserted to have been filed on behalf of the true party in interest, such that USPTO processing of the request be delayed during pending concurrent litigation.

The third party petition is dismissed.

Third party petitioner who is asserted to be a defendant in litigation (Nichols Institute Diagnostics Inc. v. Scantibodies Clinical Laboratory, Inc., Civ. No. 02cv0046-B (LAB) filed May 15, 2002) involving this patent, opposes the above-noted request for a Certificate of Correction and seeks that it be refused by the USPTO.

Nevertheless, a third party does not have standing to request that the USPTO refuse to issue a Certificate of Correction. See Hallmark Cards, Inc. v. Lehman, 959 F. Supp. 539, 42 USPQ2d 1134 (D.D.C. 1997). Rather, any remedy that may be forthcoming to petitioner in this matter is more properly sought by petitioner before the court in the litigation already in progress. Id.; see also See Southwest Software, Inc. v. Harlequin Inc., 226 F.3d 1280, 56 USPQ2d 1161 (Fed. Cir. 2000). Furthermore, petitioner is not unduly prejudiced in this matter as the propriety of any change of inventorship effectuated by a Certificate of Correction is subject to judicial review. See Borden v. Occidental Petroleum Corp., 381 F.Supp. 1178, 1207, 182 USPQ 472 (S.D. Tex. 1974). Thus, when the requirements of 35 U.S.C. § 256 are met by the inventors, it is appropriate for the Director of the USPTO to perform the duty to issue the certificate in the ordinary way without delay. If the District Court reaches a different result on the merits, it may order correction under 35 U.S.C. § 256.

Lastly, a standard principle of statutory construction is: *expressio unius est exclusion*

alterius (the mention of one thing implies exclusion of another thing). See National R.R. Passenger Corp. v. National Ass'n of R.R. Passengers, 414 U.S. 453, 458 (1974); see also Botany Worsted Mills v. United States, 278 U.S. 282, 289 (1929)("when a statute limits a thing to be done in a particular mode, it includes the negative of any other mode"). As the patent statute (35 U.S.C. § 301) specifically states what submissions by third parties may be placed in the file of a patent, the patent statute implicitly excludes other third party submissions, such as that herein, from being placed in the file of a patent. Accordingly, the third party papers are being returned herewith. Cf. Ex Parte Chambers et al., 20 USPQ 1470 (Comm'r Pat. 1991); In re Dubno, 12 USPQ2d 1153 (Comm'r Pat. 1989).

This patent file is being forwarded to Certificates of Correction Division.

Telephone inquiries concerning this decision may be directed to the undersigned at (703) 305-1820.

Brian Hearn

Brian Hearn
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cc
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Enclosure for cc: Papers filed July 15, 16, 19, 2002

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SCANTIBODIES CLINICAL LABORATORY, INC. and SCANTIBODIES LABORATORY, INC.

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

NICHOLS INSTITUTE DIAGNOSTICS, INC., a California corporation,

Plaintiff,

v.

SCANTIBODIES CLINICAL LABORATORY, INC., a California corporation; and SCANTIBODIES LABORATORY, INC., a California corporation,

Defendants.

SCANTIBODIES CLINICAL LABORATORY, INC., a California corporation; and SCANTIBODIES LABORATORY, INC., a California corporation,

Counter-Claimants

v.

NICHOLS INSTITUTE DIAGNOSTICS, INC., a California corporation,

Counter-Defendants.

No. 02 CV 0046 B (LAB)

DECLARATION OF M. ANDREW WOODMANSEE IN SUPPORT OF SCANTIBODIES CLINICAL LABORATORY, INC. AND SCANTIBODIES LABORATORY, INC.'S MOTION FOR SUMMARY JUDGMENT PURSUANT TO 35 U.S.C. § 102(f) FOR NONJOINDER OF CO-INVENTOR

Date: July 15, 2002
Time: 10:30 a.m.
Courtroom 2

Hon. Rudi M. Brewster

I, M. Andrew Woodmansee, declare:

1. I am a member of the bar of the State of California and am with the law firm of Morrison & Foerster LLP, which represents Scantibodies Clinical Laboratory, Inc. and Scantibodies Laboratory, Inc. ("Scantibodies") in this matter. I have personal knowledge of the facts stated herein and, if called as a witness, I could and would testify competently as to them.

2. Attached as Exhibit A to this declaration is a true and correct copy of a certified translation from German to English of German patent number DE 44 34 555 A1.

3. Attached as Exhibit B to this declaration is a true and correct copy of a certified translation from German to English of an international patent application (WO 96/10041) filed with the World Intellectual Property Organization and published pursuant to the Patent Cooperation Treaty ("PCT WO 96/10041").

4. Attached as Exhibit C to this declaration is a true and correct copy of U.S. Patent No. 6,030,790.

5. Attached as Exhibit D to this declaration is a copy of the initial U.S. Patent Application Serial No. 08/817547 and declarations filed with the United States Patent and Trademark Office, which issued as Patent No. 6,030,790.

6. Attached as Exhibit E to this declaration is a true and correct copy of the untranslated German patent number DE 44 34 555 A1.

7. Attached as Exhibit F to this declaration is a true and correct copy of the untranslated PCT Application WO 96/10041.

I declare under penalty of perjury under the laws of the United State that the foregoing is true and correct. Executed this 15 day of May 2002, at San Diego, California.

M. Andrew Woodmansee

Exhibit A

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TRANSLATIONS

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Certificate of Accuracy

This is to certify that the attached document, Patent DE 44 34 551 A1, originally written in German is, to the best of our knowledge and belief, a true, accurate and complete translation into English.

Dated: May 13, 2002

Jeffrey Mamera
Director, Translations
Merrill Corporation

Sworn to and signed before
Me this 13th day of
May, 2002

Notary Public

THOMAS C. ALWOOD
Notary Public, State of New York
No. 01AL6004438 Qualified in Kings County
Certificate Filed in New York County
Commission Expires: 3/26/2006

19. **Federal Republic of Germany**
German Patent Office

51. Int. Cl.[6]: **C 07 K 14/635**

12. **Disclosure Document**
11. **DE 44 34 551 A 1**

DE 44 34 551 A1

21. File No.: P 44 34 551.8
22. Date of Application: 9/28/94
43. Date of Disclosure: 4/4/96

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Examination application filed pursuant to § 44, PatG [Patent Law].

54. Peptides from the hPTH Sequence (1-37)

57. The invention relates to peptides from the human parathyroid (hPTH) sequence (1-37), containing α-helical amino acid sequence regions and/or unstructured amino acid sequence regions, where said peptides are capable of inducing antibodies when injected into animals. The invention also relates to a diagnostic agent and antibodies obtainable by vaccination of animals with the peptides in question.

DE 197 33 666 A 1

The following information is taken from documentation filed by the Applicant.

Peptides from the hPTH Sequence (1-37)

This invention relates to peptides from the hPTH sequence (1-37), a diagnostic agent obtainable by vaccination of animals with the peptides, antibodies or fragments thereof, that can be obtained by vaccination of animals with the peptides, as well as the use of peptides for production of an agent for diagnosis of biologically active h-PTH.

Human parathyroid hormone (hPTH), a linear polypeptide of 84 amino acids, plays an important role in regulating the calcium metabolism. The metabolism of this hormone leads to a large number of C-terminal fragments whose biological function has not been clarified yet. Human PTH 1-37 has been detected as a circulating N-terminal fragment (EP-A 0 349 545). This fragment possesses the full biological activity of the complete hormone. This decreases substantially, however, upon loss of the first amino acid, serine, and is completely lost upon removal of the first two amino acids, serine and valine.

For the intact hormone, hPTH 1-84, and for the N-terminal fragment, serum concentrations have been measured in the area of 10^{-12} mol/L. Immunological measurement techniques are used to detect such low concentrations. The most valid results are provided by the double-antibody or sandwich principle (e.g., two-site immunoradiometric assay (IRMA), or Sandwich Enzyme Linked Immunosorbent Assay (Sandwich ELISA)). These assays for hPTH 1-84 are available commercially. There is no assay for hPTH 1-34 using the double antibody principle.

Two antibodies are required for this. To avoid reciprocal steric hindrance, the method must recognize epitopes of the antigen located at a sufficient distance from each other. Vaccination with intact antigens results in a heterogeneous mixture of various antibodies that have to be purified before the sandwich assay. Of course, detecting a preferred immunoactive sequence in the region of amino acids 7-14 at the N terminus was possible previously based on theoretical calculations according to B.A. Jameson & H., Wolf, "The Antigenic Index: A Novel Algorithm for Predicting Antigenic Determinants", CABIOS 4, pp. 181-186, 1988. Vaccination with N-terminal fragments according to established methods leads, initially, to antibodies that bind in this amino acid region, as described for hPTH 1-34 (J. Tampe, P. Brozio, H.E. Manneck, A. Mißbichler, E. Blind, K.B. Müller, H. Schmidt-Gayk and F.P. Armbruster; "Characterization of Antibodies Against Human N-Terminal Parathyroid Hormone by Epitope Mapping"; J. Immunoassay 13, pp. 1-13, 1992). However, these antibodies cannot distinguish between biologically-active and biologically-inactive PTH 1-84 or fragments thereof missing the first two amino acids, serine and valine.

The problem to which the invention relates consists of specifying peptides that can help to eliminate the above disadvantages in diagnosing biologically-active h-PTH.

The technical problem discussed is solved surprisingly by peptides from the hPTH sequence (1-37) containing α-helical amino acid sequence regions and/or unstructured amino acid sequence regions, where vaccination of animals with the peptides can induce antibodies. The peptides here preferably contain the N terminal α helix in the region of amino acids 5-9, an unstructured section of amino acids 10-16 and/or a C terminal α helix in the region of amino acid sequence 17-34 of the hPTH (1-37). The following peptides according to the invention are preferably used for the vaccination:

hPTH 1-10 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH$ (1)
hPTH 1-9 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH$ (2)
hPTH 1-8 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH$ (3)
hPTH 1-7 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH$ (4)
hPTH 1-6 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH$ (5)
hPTH 1-5 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH$ (6)
hPTH 9-18 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (7)
hPTH 10-18 $NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (8)
hPTH 11-18 $NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (9)
hPTH 12-18 $NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (10)
hPTH 13-18 $NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (11)
hPTH 14-18 $NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (12)
hPTH 9-17 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH$ (13)
hPTH 9-16 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH$ (14)
hPTH 9-15 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH$ (15)
hPTH 9-14 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH$ (16)
hPTH 9-13 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH$ (17)
hPTH 24-37
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (18)
hPTH 25-37
$NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (19)
hPTH 26-37
$NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (20)
hPTH 27-37
$NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (21)

hPTH 28-37 $NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (22)
hPTH 29-37 $NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (23)
hPTH 30-37 $NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (24)
hPTH 31-37 $NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (25)
hPTH 32-37 $NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (26)
hPTH 33-37 $NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (27)
hPTH 24-36
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH$ (28)
hPTH 24-35
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH$ (29)
hPTH 24-34
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH$ (30)
hPTH 24-33 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}OH$ (31)
hPTH 24-32 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH$ (32)
hPTH 24-31 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH$ (33)
hPTH 24-30 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-OH$ (34)
hPTH 24-29 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH$ (35)
hPTH 24-28 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH$ (36)

The primary structures of the above sequences represent essential characteristics of the secondary structure, as supported by the NMR data. A precondition for this was determining the secondary structure of PTH 1-37 in saline solution.

The structurally-remarkable regions cited have good immunogenic activity. Antibodies are formed that bind to the first amino acids of the N terminus. The lack of two amino acids already leads to a substantial loss of affinity. Since these amino acids are essential to biological activity, it is possible to obtain antibodies with the peptides according to the invention that recognize only hPTH and fragments thereof that are biologically active.

Furthermore, antibodies can be produced that detect the mid-region areas 9-15, and antibodies that bind the L terminal in the region of amino acids 30-37. According to the invention, therefore, antibodies can be produced against regions of hPTH 1-37 that do not have immunogenic effects based on theoretical calculations in the intact molecule. These regions are also located at such a distance from each other that there is no steric hindrance that would hinder the simultaneous binding of two antibodies.

In the preferred embodiment, the peptides can be modified at the N terminal end, the side chains and/or at the C terminal end, by acetylation, amidation, phosphorylation and/or glycosylation products.

Finally, peptides according to the invention can also be bound to carrier proteins such as hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin or mouse serum albumin. They are preferably bound with the carrier protein through carbodiimide or formaldehyde.

The peptides according to the invention can be used to produce a diagnostic agent. The diagnostic agent according to the invention can be obtained by known vaccination of animals with at least one of the peptides according to the invention. After vaccination, an immunoglobulin fraction can be isolated from the vaccinated animals; the fraction contains antibody fractions that have an antibody titer against at least one of the peptides according to the invention. The antibodies thus obtained are also the subject of this invention. In an alternative embodiment, in addition to the complete antibodies consisting of F_{ab} and F_c, fragments thereof, such as F_{ab}, or fragments of antibodies are used; they are the idiotypes for the epitopes of the peptides.

The peptides according to the invention are suitable for production of an agent for diagnosing biologically-active h-PTH (1-37).

The invention is described in greater detail based on the following examples:

Example 1

Solid Phase Peptide Synthesis

The process for synthesis of peptides according to the invention is based on peptide synthesis on solid carriers. The C terminal amino acids are bound to the carrier material in the presence of dicyclohexylcarbodiimide and dimethylaminopyridine. The carrier material used for the synthesis is Wang resin or similar resins.

The following L-amino acid derivatives are used for the synthesis of the sequence, starting with the specified peptidyl resin: a) hPTH 1-10: Fmoc-Asn(Trt)-Wang resin, Fmoc-His(Trt)-OH, Fmoc-Met-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Bocer(tBu)-OH. b) hPTH 9-18: Fmoc-Met-Wang resin, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Boc-His(Trt)-OH. c) hPTH 24-37: Fmoc-Leu-Wang resin, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Asn(Trt)-OH, Fmoc-His(Trt)-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH.

The synthesis can be carried out through in-situ activation with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or derivatives thereof, or with benzotriazol-1-yloxy-(trisdimethylamino)phosphonium hexafluorophosphate (BOP) or derivatives thereof, in the presence of diisopropyl ethylamine or N-methylmorpholine and 1-hydroxybenzotriazole, where a four- to ten-fold excess of Fmoc-L-amino acid is used during the couplings in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. The Fmoc groups are dissociated with 20% piperidine or 2% piperidine and 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. After synthesis, the resin is washed with 2-propanol and dichloromethane and dried to a constant weight under a high vacuum.

For dissociation from the carrier and unblocking, the peptidyl resin is treated for 30 – 90 minutes at ambient temperature with trifluoroacetic acid containing 5% scavenger, water, ethanediol, phenol or thioanisole, then filtered, washed with trifluoroacetic acid and finally precipitated with tert-butyl methyl ether. The precipitate is lyophilized out of an aqueous solution.

Example 2

Purification and Analysis

The raw product is purified by chromatography in a C-18 reverse phase column (10 µm, Buffer A: 0.01 N HCl in water; Buffer B: 20% isopropenol, 30% methanol, 50% water, 0.01 N HCl; gradient: 10 – 80% in 60 minutes; detection 230 nm).

The purity of the product is determined by mass spectrometry and C18 reverse phase chromatography.

Example 3

Coupling to Carrier Protein

Hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin or mouse serum albumin is used as a carrier protein. The coupling takes place according to the carbodiimide method, via the carboxyl groups of the peptide. The peptide is activated by a 5-minute treatment in an aqueous solution with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. Coupling occurs by the addition of the activated peptide to an aqueous solution of the carrier. The molar ratio is 1 peptide to 50 amino acids of the carrier protein. The treatment takes 4 hours.

The reaction is stopped by addition of sodium acetate in a final concentration of 100 mM. Incubate for 1 hour.

The protein-peptide conjugate is separated from the peptide by repeated dialysis over 100 mM of phosphate buffer, pH 7.2

Example 4

Synthesis of Multiple Antigenic Peptides (MAP)

The triple lysine branch is achieved by binding Fmoc-L-lysine(Fmoc)-OH to C terminal alanine, bound to Wang resin, in three coupling cycles. Eight free amino functions are obtained by dissociation with piperidine; the sequences of human parathyroid hormone are synthesized at those functions according to the description above.

Example 5

Vaccination

125 µg of the carrier-peptide conjugate or MAP, dissolved in 250 ml water and emulsified with 250 µl of complete Freund's adjuvant, is used per kg of body weight for the first vaccination. The emulsion is applied to the back in 10 separate SC injections.

Boosters are given similarly after 2-4 weeks. However, the complete Freund's adjuvant is replaced with incomplete Freund's adjuvant here.

Patent Claims

We claim:

1. A peptide from the hPTH sequence (1-37) containing α-helical amino acid sequence regions and/or unstructured amino acid sequence regions, wherein the peptides can induce antibodies upon injection into animals.

2. Peptides according to Claim 1, from hPTH (1-37), with the sequence:

hPTH 1-10 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH$ (1)
hPTH 1-9 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH$ (2)
hPTH 1-8 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH$ (3)
hPTH 1-7 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH$ (4)
hPTH 1-6 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH$ (5)
hPTH 1-5 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH$ (6)
hPTH 9-18 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (7)
hPTH 10-18 $NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (8)
hPTH 11-18 $NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (9)
hPTH 12-18 $NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (10)
hPTH 13-18 $NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (11)
hPTH 14-18 $NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (12)
hPTH 9-17 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH$ (13)
hPTH 9-16 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH$ (14)
hPTH 9-15 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH$ (15)
hPTH 9-14 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH$ (16)
hPTH 9-13 $TH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH$ (17)
hPTH 24-37
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (18)
hPTH 25-37
$NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (19)
hPTH 26-37
$NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (20)
hPTH 27-37
$NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (21)
hPTH 28-37
$NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (22)
hPTH 29-37 $NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (23)
hPTH 30-37 $NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (24)
hPTH 31-37 $NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (25)
hPTH 32-37 $NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (26)
hPTH 33-37 $NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (27)
hPTH 24-36
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH$ (28)
hPTH 24-35
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH$ (29)
hPTH 24-34
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH$ (30)
hPTH 24-33
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH$ (31)
hPTH 24-32 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH$ (32)
hPTH 24-31 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH$ (33)
hPTH 24-30 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-OH$ (34)
hPTH 24-29 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH$ (35)
hPTH 24-28 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH$ (36).

3. Peptides according to Claim 1 and/or 2, modified at the N-terminal end, the side chains and/or the C-terminal end with acetylation, amidation, phosphorylation and/or glycosylation products, and/or bound to carrier proteins such as hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin or mouse serum albumin.

4. A diagnostic agent obtainable by vaccination, known *per se*, of animals with at least one of the peptides according to at least one of the Claims 1 through 3, by obtaining fractions containing immunoglobulins from the vaccinated animals and by isolating fractions with an antibody titer against at least one of the peptides according to at least one of Claims 1 through 3 and that contains, if necessary, other adjuvants and/or carriers.

5. Antibody or fragments of antibodies obtained by vaccination of animals with at least one peptide according to at least one of Claims 1 through 3.

6. Use of the peptides according to at least one of Claims 1 through 3 for production of an agent for diagnosis of biologically-active h-PTH (1-37).

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Exhibit B

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Dated: May 13, 2002

Jeffrey Mamera
Director, Translations
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Sworn to and signed before
Me this 13th day of
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(57) Abstract

The invention concerns peptides from the human parathyroid (hPTH) sequence (1-37) and containing α-helical amino acid sequence regions and/or non-structured amino acid sequence regions. The said peptides are capable of inducing antibodies when injected into animals. The invention also concerns a diagnostic agent and antibodies obtainable by vaccination of animals with the peptides in question.

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Peptides from the hPTH Sequence (1-37)

This invention relates to peptides from the hPTH sequence (1-37), a diagnostic agent obtainable by vaccination of animals with the peptides, antibodies or fragments thereof, that can be obtained by vaccination of animals with the peptides, as well as the use of peptides for production of an agent for diagnosis of biologically active h-PTH.

Human parathyroid hormone (hPTH), a linear polypeptide of 84 amino acids, plays an important role in regulating the calcium metabolism. The metabolism of this hormone leads to a large number of C-terminal fragments whose biological function has not been clarified yet. Human PTH 1-37 has been detected as a circulating N-terminal fragment (EP-A 0 349 545). This fragment possesses the full biological activity of the complete hormone. This decreases substantially, however, upon loss of the first amino acid, serine, and is completely lost upon removal of the first two amino acids, serine and valine.

For the intact hormone, hPTH 1-84, and for the N-terminal fragment, serum concentrations have been measured in the area of 10^{-12} mol/L. Immunological measurement techniques are used to detect such low concentrations. The most valid results are provided by the double-antibody or sandwich principle (e.g., two-site immunoradiometric assay (IRMA), or Sandwich Enzyme Linked Immunosorbent Assay (Sandwich ELISA)). These assays for hPTH 1-84 are available commercially. There is no assay for hPTH 1034 using the double antibody principle.

Two antibodies are required for this. To avoid reciprocal steric hindrance, the method must recognize epitopes of the antigen located at a sufficient distance from each other. Vaccination with intact antigens results in a heterogeneous mixture of

various antibodies that have to be purified before the sandwich assay. Of course, detecting a preferred immunoactive sequence in the region of amino acids 7-14 at the N terminus was possible previously based on theoretical calculations according to B.A. Jameson & H., Wolf, "The Antigenic Index: A Novel Algorithm for Predicting Antigenic Determinants", CABIOS 4, pp. 181-186, 1988. Vaccination with N-terminal fragments according to established methods leads, initially, to antibodies that bind in this amino acid region, as described for hPTH 1-34 (J. Tampe, P. Brozio, H.E. Manneck, A. Mißbichler, E. Blind, K.B. Müller, H. Schmidt-Gayk and F.P. Armbruster; "Characterization of Antibodies Against Human N-Terminal Parathyroid Hormone by Epitope Mapping"; J. Immunoassay 13, pp. 1-13, 1992). However, these antibodies cannot distinguish between biologically-active and biologically-inactive PTH 1-84 or fragments thereof missing the first two amino acids, serine and valine.

The problem to which the invention relates consists of specifying peptides that can help to eliminate the above disadvantages in diagnosing biologically-active h-PTH.

The technical problem discussed is solved surprisingly by peptides from the hPTH sequence (1-37) containing α-helical amino acid sequence regions and/or unstructured amino acid sequence regions, where vaccination of animals with the peptides can induce antibodies. The peptides here preferably contain the N terminal α helix in the region of amino acids 5-9, an unstructured section of amino acids 10-16 and/or a C terminal α helix in the region of amino acid sequence 17-34 of the hPTH (1-37). The following peptides according to the invention are preferably used for the vaccination:

hPTH 1-10

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18

NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18

NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18

NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18

NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18

NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18

NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 9-17
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

hPTH 9-16
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

hPTH 9-15
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

hPTH 9-14
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

hPTH 9-13
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

hPTH 24-37
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

hPTH 25-37
NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

hPTH 26-37
NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

hPTH 27-37
NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

hPTH 28-37
NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (22)

hPTH 29-37
NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

hPTH 30-37

NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

hPTH 31-37

NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

hPTH 32-37

NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

hPTH 33-37

NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

hPTH 24-36

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

hPTH 24-35

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

hPTH 24-34

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

hPTH 24-33

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

hPTH 24-32

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

hPTH 24-31

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

hPTH 24-30

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-OH (34)

hPTH 24-29
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (35)

hPTH 24-28
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (36)

The primary structures of the above sequences represent essential characteristics of the secondary structure, as supported by the NMR data. A precondition for this was determining the secondary structure of PTH 1-37 in saline solution.

The structurally-remarkable regions cited have good immunogenic activity. Antibodies are formed that bind to the first amino acids of the N terminus. The lack of two amino acids already leads to a substantial loss of affinity. Since these amino acids are essential to biological activity, it is possible to obtain antibodies with the peptides according to the invention that recognize only hPTH and fragments thereof that are biologically active.

Furthermore, antibodies can be produced that detect the mid-region areas 9-15, and antibodies that bind in the region of amino acids 30-37. According to the invention, therefore, antibodies can be produced against regions of hPTH 1-37 that do not have immunogenic effects based on theoretical calculations in the intact molecule. These regions are also located at such a distance from each other that there is no steric hindrance that would hinder the simultaneous binding of two antibodies.

In the preferred embodiment, the peptides can be modified at the N terminal end, the side chains and/or at the C terminal end, by acetylation, amidation, phosphorylation and/or glycosylation products.

Finally, peptides according to the invention can also be bound to carrier proteins such as hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin or mouse serum albumin. They are preferably bound with the carrier protein through carbodiimide or formaldehyde.

The peptides according to the invention can be used to produce a diagnostic agent. The diagnostic agent according to the invention can be obtained by known vaccination of animals with at least one of the peptides according to the invention. After vaccination, an immunoglobulin fraction can be isolated from the vaccinated animals; the fraction contains antibody fractions that have an antibody titer against at least one of the peptides according to the invention. The antibodies thus obtained are also the subject of this invention. In an alternative embodiment, in addition to the complete antibodies consisting of F_{ab} and F_c, fragments thereof, such as F_{ab}, or fragments of antibodies are used; they are the idiotypes for the epitopes of the peptides.

The peptides according to the invention are suitable for production of an agent for diagnosing biologically-active h-PTH (1-37).

The invention is described in greater detail based on the following examples:

Example 1

<u>Solid Phase Peptide Synthesis</u>

The process for synthesis of peptides according to the invention is based on peptide synthesis on solid carriers. The C terminal amino acids are bound to the carrier material in the presence of

dicyclohexylcarbodiimide and dimethylaminopyridine. The carrier material used for the synthesis is Wang resin or similar resins.

The following L-amino acid derivatives are used for the synthesis of the sequence, starting with the specified peptidyl resin:
a) hPTH 1-10: Fmoc-Asn(Trt)-Wang resin, Fmoc-His(Trt)-OH, Fmoc-Met-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Boc-Ser(tBu)-OH.
b) hPTH 9-18: Fmoc-Met-Wang resin, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Boc-His(Trt)-OH.
c) hPTH 24-37: Fmoc-Leu-Wang resin, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Asn(Trt)-OH, Fmoc-His(Trt)-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH.

The synthesis can be carried out through in-situ activation with 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or derivatives thereof, or with benzotriazol-1-yloxy-trisdimethylamino phosphonium hexafluorophosphate (BOP) or derivatives thereof in the presence of diisopropyl ethylamine or N-methylmorpholine and 1-hydroxybenzotriazole, where a four- to ten-fold excess of Fmoc-L-amino acid is used during the couplings in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. The Fmoc groups are dissociated with 20% piperidine or 2% piperidine and 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. After synthesis, the resin is washed with 2-propanol and dichloromethane and dried to a constant weight under a high vacuum.

For dissociation from the carrier and unblocking, the peptidyl resin is treated for 30 - 90 minutes at ambient temperature with trifluoroacetic acid containing 5% scavenger, water, ethanediol,

phenol or thioanisole, then filtered, washed with trifluoroacetic acid and finally precipitated with tert-butyl methyl ether. The precipitate is lyophilized out of an aqueous solution.

Example 2

Purification and Analysis

The raw product is purified by chromatography in a C-18 reverse phase column (10 µm, Buffer A: 0.01 N HCl in water; Buffer B: 20% isopropenol, 30% methanol, 50% water, 0.01 N HCl; gradient: 10 - 80% in 60 minutes; detection 230 nm).

The purity of the product is determined by mass spectrometry and C18 reverse phase chromatography.

Example 3

Coupling to Carrier Protein

Hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin or mouse serum albumin is used as a carrier protein. The coupling takes place according to the carbodiimide method, via the carboxyl groups of the peptide. The peptide is activated by a 5-minute treatment in an aqueous solution with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride. Coupling occurs by the addition of the activated peptide to an aqueous solution of the carrier. The molar ratio is 1 peptide to 50 amino acids of the carrier protein. The treatment takes 4 hours.

The reaction is stopped by addition of sodium acetate in a final concentration of 100 mM. Incubate for 1 hour.

The protein-peptide conjugate is separated from the peptide by repeated dialysis over 100 mM of phosphate buffer, pH 7.2

Example 4

Synthesis of Multiple Antigenic Peptides (MAP)

The triple lysine branch is achieved by binding Fmoc-L-lysine(Fmoc)-OH to C terminal alanine, bound to Wang resin, in three coupling cycles. Eight free amino functions are obtained by dissociation with piperidine; the sequences of human parathyroid hormone are synthesized at those functions according to the description above.

Example 5

Vaccination

125 µg of the carrier-peptide conjugate or MAP, dissolved in 250 ml water and emulsified with 250 µl of complete Freund's adjuvant, is used per kg of body weight for the first vaccination. The emulsion is applied to the back in 10 separate SC injections.

Boosters are given similarly after 2-4 weeks. However, the complete Freund's adjuvant is replaced with incomplete Freund's adjuvant here.

Patent Claims

We claim:

1. A peptide from the hPTH sequence (1-37) containing α-helical amino acid sequence regions and/or unstructured amino acid sequence regions, wherein the peptides can induce antibodies upon injection into animals.

2. Peptides according to Claim 1, from hPTH (1-37), with the sequence:

hPTH 1-10
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18
NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18

NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18

NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18

NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18

NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18

NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 9-17

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

hPTH 9-16

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

hPTH 9-15

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

hPTH 9-14

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

hPTH 9-13

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

hPTH 24-37

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

hPTH 25-37

NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

hPTH 26-37

NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

hPTH 27-37

NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

hPTH 28-37

NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (22)

hPTH 29-37

NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

hPTH 30-37

NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

hPTH 31-37

NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

hPTH 32-37

NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

hPTH 33-37

NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

hPTH 24-36

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

hPTH 24-35

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

hPTH 24-34

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

hPTH 24-33

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

hPTH 24-32

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

hPTH 24-31

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

hPTH 24-30

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-OH (34)

hPTH 24-29

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (35)

hPTH 24-28

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (36)

3. Peptides according to Claim 1 and/or 2, modified at the N-terminal end, the side chains and/or the C-terminal end with acetylation, amidation, phosphorylation and/or glycosylation products, and/or bound to carrier proteins such as hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin or mouse serum albumin.

4. A diagnostic agent obtainable by vaccination, known *per se*, of animals with at least one of the peptides according to at least one of the Claims 1 through 3, by obtaining fractions containing immunoglobulins from the vaccinated animals and by isolating fractions with an antibody titer against at least one of the peptides according to at least one of Claims 1 through 3 and that contains, if necessary, other adjuvants and/or carriers.

5. Antibody or fragments of antibodies obtained by vaccination of animals with at least one peptide according to at least one of Claims 1 through 3.

6. Use of the peptides according to at least one of Claims 1 through 3 for production of an agent for diagnosis of biologically-active h-PTH (1-37).

[International Search Report in English]

[International Search Report in German identical to English-language Search Report]

Exhibit C

US006030790A

United States Patent [19]

Adermann et al.

[11] Patent Number: 6,030,790

[45] Date of Patent: Feb. 29, 2000

[54] ANTIBODIES THAT BIND PEPTIDES FROM THE HPTH SEQUENCE (1-37)

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[21] Appl. No.: 08/817,547

[22] PCT Filed: Sep. 22, 1995

[86] PCT No.: PCT/EP95/03757

§ 371 Date: Mar. 27, 1997

§ 102(e) Date: Mar. 27, 1997

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[30] Foreign Application Priority Data

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[51] Int. Cl.[7] G01N 33/43; C07K 16/26

[52] U.S. Cl. 435/7.1; 436/512; 530/387.1; 530/387.2; 530/387.9; 530/388.24

[58] Field of Search 435/7.1; 436/512; 530/387.1, 387.2, 387.9, 388.24

[56] References Cited

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Primary Examiner—Elizabeth Kemmerer

Attorney, Agent, or Firm—Jones & Askew, LLP

[57] ABSTRACT

The present invention is directed to peptides from the sequence of hPTH(1–37), which contain α-helical amino acid sequence regions and/or non-structured amino acid sequence regions, said peptides being capable of inducing antibodies when injected into animals. Furthermore, the invention is directed to a diagnostic agent and antibodies obtainable by immunizing animals using the peptides according to the invention.

25 Claims, No Drawings

ANTIBODIES THAT BIND PEPTIDES FROM THE HPTH SEQUENCE (1-37)

This application was filed under 35 U.S.C. § 371 and claims priority from PCT/EP95/03757, filed Sep. 22, 1995.

The present invention relates to peptides from the sequence of hPTH(1–37), and the use of said peptides in the preparation of an agent for diagnosing biologically active hPTH.

Human parathyroid hormone (hPTH), a linear polypeptide having 84 amino acids, plays an important role in the regulation of the calcium metabolism. The metabolism of this hormone gives rise to a large number of C-terminal fragments, the biological functions of which have not yet been elucidated. The hPTH(1–37) has been established as a circulating N-terminal fragment (EP-A 0 349 545). This fragment has the full biological activity of the entire hormone. However, upon loss of the first amino acid, serine, the activity significantly decreases and is lost completely without the first two amino acids, serine and valine.

Serum levels in the range of 10^{-12} mol/l are measured for the intact hormone hPTH(1–84) and for the N-terminal fragment. Immunological measuring procedures are employed to determine such low concentrations. Here, the most valid results are provided by measuring procedures according to the double antibody or sandwich principle (e.g., the two-site radioimmunometric assay, IRMA, or the sandwich enzyme-linked immuno sorbent assay, Sandwich ELISA). For hPTH(1–84), such assays are commercially available. For the measurement of hPTH(1–34), an assay according to the double antibody principle is not known.

Here, two antibodies are required. In order to avoid mutual steric hindrance, they must be capable of recognizing antigen epitopes located at a sufficient distance from each other. When immunizing using the intact antigen, a heterogeneous mixture of various antibodies is obtained, which first must be subjected to an expensive purification in order to conduct a sandwich assay. According to theoretical calculations by B. A. Jameson and H. Wolf, The Antigenic Index: A Novel Algorithm for Predicting Antigenic Determinants, CABIOS 4, p. 181–186, 1988; it has been possible so far to detect a preferred sequence having immunogenic activity in the region of the amino acids 7–14 at the N-terminus. Immunization with N-terminal fragments according to established methods predominantly results in antibodies which, as has been described for hPTH(1–34) (J. Tampe, P. Brozio, H. E. Manneck, A. Miβbichler, E. Blind, K. B. Millers, H. SchmidtGayk, and F. P. Armbruster, Characterisation of Antibodies Against Human N-Terminal Parathyroid Hormone by Epitope Mapping; J. Immunoassay 13, p. 1–13, 1992), bind in the region of these amino acids. However, these antibodies are not capable of discriminating between biologically active and biologically inactive PTH (1–84) or fragments thereof lacking the first two amino acids serine and valine.

The technical problem which this invention is based upon is to provide peptides by means of which it is possible to eliminate the above-mentioned drawbacks in the diagnosis of biologically active hPTH.

Surprisingly, the technical problem described above is solved by means of the following peptides from the sequence of hPTH(1–37):

hPTH 1-10 SEQ I.D. NO.1
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9 SEQ I.D. NO.2
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8 SEQ I.D. NO.3
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7 SEQ I.D. NO.4
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6 SEQ I.D. NO.5
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5 SEQ I.D. NO.6
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18 SEQ I.D. NO.7
NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18 SEQ I.D. NO.8
NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18 SEQ I.D. NO.9
NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18 SEQ I.D. NO.10
NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18 SEQ I.D. NO.11
NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18 SEQ I.D. NO.12
NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 9-17 SEQ I.D. NO.13
NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

hPTH 9-16 SEQ I.D. NO.14

-continued

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

hPTH 9-15 SEQ I.D. NO.15
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

hPTH 9-14 SEQ I.D. NO.16
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

hPTH 9-13 SEQ I.D. NO.17
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

hPTH 24-37 SEQ I.D. NO.18
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

hPTH 25-37 SEQ I.D. NO.19
NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

hPTH 26-37 SEQ I.D. NO.20
NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

hPTH 27-37 SEQ I.D. NO.21
NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

hPTH 28-37 SEQ I.D. NO.22
NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (22)

hPTH 29-37 SEQ I.D. NO.23
NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

hPTH 30-37 SEQ I.D. NO.24
NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

hPTH 31-37 SEQ I.D. NO.25
NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

hPTH 32-37 SEQ I.D. NO.26
NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

hPTH 33-37 SEQ I.D. NO.27
NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

hPTH 24-36 SEQ I.D. NO.28
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

hPTH 24-35 SEQ I.D. NO.29
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

hPTH 24-34 SEQ I.D. NO.30
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

hPTH 24-33 SEQ I.D. NO.31
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

hPTH 24-32 SEQ I.D. NO.32
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

hPTH 24-31 SEQ I.D. NO.33
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

hPTH 24-29 SEQ I.D. NO.34
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (34)

hPTH 24-38 SEQ I.D. NO.35
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (35)

The indicated sequences represent essential features of the secondary structure in their primary structure, as can be demonstrated by supporting NMR data. One precondition to this end was a determination of the PTH(1–37) secondary structure in physiological solution.

The above-mentioned regions of conspicuous structure have good immunogenic activity. Antibodies are formed, binding to the first amino acids of the N-terminus. Deficiency of only two amino acids gives rise to a substantial loss in affinity. Because these amino acids are indispensable

for the biological activity to arise, it is possible by using the peptides of the invention to obtain antibodies recognizing only hPTH and fragments thereof which are biologically active.

Furthermore, antibodies can be produced which detect the mid-region 9–15, as well as antibodies giving C-terminal binding in the region of the amino acids 30–37. According to the invention, it is therefore possible to produce antibodies against hPTH(1–37) regions which, according to theoretical calculations, do not exhibit immunogenic activity within the entire molecule. In addition, these regions are separated from each other by such a far distance that no steric hindrance is present which would prevent simultaneous binding of two antibodies.

In preferred embodiments, the peptides may be modified at the N-terminal end, in the side-chain and/or at the C-terminal end, namely, taking the form of acetylation, amidation, phosphorylation and/or glycosylation products.

Eventually, the peptides of the invention may also be bound to carrier proteins such as hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin, or mouse serum albumin etc. Binding to the carrier proteins is preferably effected using carbodiimide or formaldehyde.

The peptides of the invention may be used in the preparation of a diagnostic agent. The diagnostic agent of the invention can be obtained using the per se known immunization of animals with at least one of the peptides according to the invention. Following immunization, an immunoglobulin fraction can be isolated from the immunized animals, which contains antibody fractions having an antibody titer against at least one of the peptides of the invention. The invention is also directed to the antibodies thus obtained. In addition to the complete antibodies consisting of F_{ab} and F_c, fragments thereof such as F_{ab} or fragments of the antibodies being idiotypes of peptide epitopes may also be used in an alternative embodiment.

The peptides according to the invention are suitable for preparing an agent for the diagnosis of biologically active hPTH(1–37).

Referring to the following examples, the invention will be described in more detail.

EXAMPLE 1

Solid-Phase Synthesis of Peptides

The method of the invention for synthesizing the peptides is based on the peptide synthesis using a solid support. Each of the C-terminal amino acids is bound to the support material in the presence of dicyclohexylcarbodiimide and dimethylaminopyridine. Wang resin or similar resins are used as support material for the syntheses.

The following derivatives of L-amino acids are used in the synthesis of the sequence, starting from the peptidyl resin as specified: a) hPTH(1–10) Seq. I.D. No. 1: Fmoc-Asn(Trt)-Wang resin, Fmoc-His(Trt)-OH, Fmoc-Met-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Boc-Ser(tBu)-OH; b) hPTH(9–18) Seq. I.D. No. 7): Fmoc-Met-Wang resin, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Boc-His(Trt)-OH; c) hPTH(24–37) Seq. I.D. No. 18: Fmoc-Leu-Wang resin, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Asn(Trt)-OH, Fmoc-His(Trt)-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg (Pmc)-OH, Fmoc-Leu-OH.

The synthesis may be carried out by in situ activation using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or derivatives thereof, or benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) or derivatives thereof in the presence of diisopropylethylamine or N-methylmorpholine and 1-hydroxybenzotriazole, using a four- to tenfold excess of Fmoc-L-amino acid during the coupling reactions in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. Removal of the Fmoc groups is effected using 20% piperidine or 2% piperidine and 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in N,N-dimethylformamide,. N,N-dimethylacetamide or N-methylpyrrolidone. Following synthesis, the resins are washed with 2-propanol and dichloromethane and dried to constant weight in a high vacuum.

Removal from the support and deprotection are carried out by reacting the peptidyl resin with trifluoroacetic acid containing 5% scavenger, water, ethanediol, phenol or thioanisole for 30–90 minutes at room temperature, filtrating, washing with trifluoroacetic acid, and subsequently precipitating with tert-butyl methyl ether. The precipitate is lyophilized from aqueous solution.

EXAMPLE 2

Purification and Analysis

The raw products are purified by chromatography on a C18 reversed phase column (10 μm, buffer A: 0.01 N HCl in water; buffer B: 20% isopropanol, 30% methanol, 50% water, 0.01 N HCl; gradient: 10–80% within 60 minutes; detection at 230 nm).

The purity of the products is determined using mass spectrometry and C18 reversed phase chromatography.

EXAMPLE 3

Coupling to Carrier Protein

Used as carrier proteins are hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin, or mouse serum albumin. Coupling is performed according to the carbodiimide method by way of the carboxyl groups of the peptides. The peptide is activated in aqueous solution by reaction with 1-ethyl-3-(3-methylaminopropyl)carbodiimide hydrochloride for 5 minutes. Coupling is effected by adding the activated peptide to an aqueous solution of the carrier. The molar ratio is 1 peptide 50 amino acids of the carrier protein. The reaction takes 4 hours.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 36

-continued

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ser Val Ser Glu Ile Gln Leu Met His Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ser Val Ser Glu Ile Gln Leu Met His
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ser Val Ser Glu Ile Gln Leu Met
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ser Val Ser Glu Ile Gln Leu
1 5

-continued

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ser Val Ser Glu Ile Gln
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Ser Val Ser Glu Ile
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

His Asn Leu Gly Lys His Leu Asn Ser Met
1 5 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Asn Leu Gly Lys His Leu Asn Ser Met
1 5

-continued

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Leu Gly Lys His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Gly Lys His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Lys His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

His Leu Asn Ser Met

-continued

1 5

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

His Asn Leu Gly Lys His Leu Asn Ser
1 5

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

His Asn Leu Gly Lys His Leu Asn
1 5

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

His Asn Leu Gly Lys His Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

-continued

His Asn Leu Gly Lys His
1 5

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

His Asn Leu Gly Lys
1 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

-continued

Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Gln Asp Val His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Asp Val His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Val His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val
1 5 10

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Leu Arg Lys Lys Leu Gln Asp Val His Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

-continued

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Leu Arg Lys Lys Leu Gln Asp Val His
1 5

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Leu Arg Lys Lys Leu Gln Asp Val
1 5

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Leu Arg Lys Lys Leu Gln Asp
1 5

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Leu Arg Lys Lys Leu Gln
1 5

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

-continued

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Leu Arg Lys Lys Leu
1 5

We claim:

1. A kit for detecting active human parathyroid hormone (hPTH) comprising a container and a first group of antibodies or antibody fragments and a second group of antibodies or antibody fragments, wherein the first group selectively binds a peptide of hPTH selected from the group consisting of peptides having SEQ. ID. Nos. 1–6 and the second group selectively binds hPTH at an epitope contained within amino acids 24 to 37.

2. The kit of claim 1, wherein the second group of antibodies or antibody fragments selectively binds a peptide of hPTH selected from the group consisting of peptides having SEQ. ID. Nos. 18–36.

3. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 1.

4. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 2.

5. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 3.

6. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 4.

7. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 5.

8. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 6.

9. An immunological method of detecting active human parathyroid hormone (hPTH) in a sample comprising:

contacting the sample with a first antibody or antibody fragment which selectively binds a peptide of hPTH selected from the group consisting of peptides having SEQ. ID. Nos. 1–6, wherein the first antibody or antibody fragment binds hPTH in the sample;

contacting the sample with a second antibody or antibody fragment which selectively binds hPTH at an epitope contained within amino acids 24 to 37; wherein the second antibody or antibody fragment binds to hPTH bound by the first antibody or antibody fragment; and

detecting the binding of the first and second antibodies or antibody fragments, wherein the binding of the first and second antibodies or antibody fragments indicates the presence of active hPTH in the sample.

10. The method of claim 9, wherein the second antibody or antibody fragment selectively binds a peptide of hPTH selected from the group consisting of peptides having SEQ. ID. Nos. 18–36.

11. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 1.

12. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 2.

13. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 3.

14. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 4.

15. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 5.

16. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 6.

17. A composition comprising an antibody or antibody fragment and a suitable carrier, wherein the antibody or antibody fragment selectively binds a peptide of human parathyroid hormone (hPTH) selected from the group consisting of peptides having SEQ. ID. Nos. 1–6.

18. The composition of claim 17, wherein the composition further comprises a second antibody or antibody fragment, wherein the second antibody or antibody fragment selectively binds hPTH at an epitope contained within amino acids 24 to 37.

19. The composition of claim 17, wherein the second antibody or antibody fragment selectively binds a peptide of hPTH selected from the group consisting of peptides having SEQ. ID. Nos. 18–36.

20. The composition of claim 17, wherein the antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 1.

21. The composition of claim 17, wherein the antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 2.

22. The composition of claim 17, wherein the antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 3.

23. The composition of claim 17, wherein the antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 4.

24. The composition of claim 17, wherein the antibody or antibody fragment selectively binds peptides pf hPTH having SEQ. ID. No. 5.

25. The composition of claim 17, wherein the antibody or antibody fragment selectivley binds peptides of hPTH having SEQ. ID. No. 6.

* * * * *

Exhibit D

PATENT APPLICATION SERIAL NO. 08/817547

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

PTO-1556
(5/87)

07426-0007
(Annex TO IPER)
PCT/EP95/03757

Peptides from the hPTH(1-37) Sequence

The present invention relates to peptides from the sequence of hPTH(1-37), and the use of said peptides in the preparation of an agent for diagnosing biologically active hPTH.

Human parathyroid hormone (hPTH), a linear polypeptide having 84 amino acids, plays an important role in the regulation of the calcium metabolism. The metabolism of this hormone gives rise to a large number of C-terminal fragments, the biological functions of which have not yet been elucidated. The hPTH(1-37) has been established as a circulating N-terminal fragment (EP-A 0 349 545). This fragment has the full biological activity of the entire hormone. However, upon loss of the first amino acid, serine, the activity significantly decreases and is lost completely without the first two amino acids, serine and valine.

Serum levels in the range of 10^{-12} mol/l are measured for the intact hormone hPTH(1-84) and for the N-terminal fragment. Immunological measuring procedures are employed to determine such low concentrations. Here, the most valid results are provided by measuring procedures according to the double antibody or sandwich principle (e.g., the two-site radioimmunometric assay, IRMA, or the sandwich enzyme-linked immuno sorbent assay, Sandwich ELISA). For hPTH(1-84), such assays are commercially available. For the measurement of hPTH(1-34), an assay according to the double antibody principle is not known.

Here, two antibodies are required. In order to avoid mutual steric hindrance, they must be capable of recognizing antigen epitopes located at a sufficient distance from each other. When immunizing using the intact antigen, a heteroge-

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neous mixture of various antibodies is obtained, which first must be subjected to an expensive purification in order to conduct a sandwich assay. According to theoretical calculations by B.A. Jameson and H. Wolf, The Antigenic Index: A Novel Algorithm for Predicting Antigenic Determinants, CABIOS 4, p. 181-186, 1988; it has been possible so far to detect a preferred sequence having immunogenic activity in the region of the amino acids 7-14 at the N-terminus. Immunization with N-terminal fragments according to established methods predominantly results in antibodies which, as has been described for hPTH(1-34) (J. Tampe, P. Brozio, H.E. Manneck, A. Mißbichler, E. Blind, K.B. Millers, H. Schmidt-Gayk, and F.P. Armbruster, Characterisation of Antibodies Against Human N-Terminal Parathyroid Hormone by Epitope Mapping; J. Immunoassay 13, p. 1-13, 1992), bind in the region of these amino acids. However, these antibodies are not capable of discriminating between biologically active and biologically inactive PTH(1-84) or fragments thereof lacking the first two amino acids serine and valine.

The technical problem which this invention is based upon is to provide peptides by means of which it is possible to eliminate the above-mentioned drawbacks in the diagnosis of biologically active hPTH.

Surprisingly, the technical problem described above is solved by means of the following peptides from the sequence of hPTH(1-37):

hPTH 1-10 SEQ. ID. NO. 1

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9 SEQ. ID. NO. 2

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8 SEQ ID NO. 3

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7 SEQ. I. D. NO. 4

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6 SEQ I. D. NO. 5

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5 SEQ. ID. NO. 6

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18 SEQ. I. D. NO. 7

NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18 SEQ. ID. NO. 8

NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18 SEQ ID. NO. 9

NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18 SEQ. I. D. NO. 10

NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18 SEQ. ID. NO. 11

NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18 SEQ. I. D. NO. 12

NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 1-10 SEQ. ID. NO. 1

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9 SEQ. ID. NO. 2

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8 SEQ. ID. NO. 3

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7 SEQ. ID. NO. 4

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6 SEQ. ID. NO. 5

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5 SEQ. ID. NO. 6

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18 SEQ. ID. NO. 7

NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18 SEQ. ID. NO. 8

NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18 SEQ. ID. NO. 9

NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18 SEQ. ID. NO. 10

NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18 SEQ. ID. NO. 11

NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18 SEQ. ID. NO. 12

NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

D hPTH 9-17 SEQ. I.D. NO 13

NH$_2$-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

D hPTH 9-16 SEQ I.D. NO 14

NH$_2$-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

D hPTH 9-15 SEQ. I.D. NO. 15

NH$_2$-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

D hPTH 9-14 SEQ. I.D. NO. 16

NH$_2$-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

D hPTH 9-13 SEQ. I.D. NO. 17

NH$_2$-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

D hPTH 24-37 SEQ. I.D. NO 18

NH$_2$-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

D hPTH 25-37 SEQ. I.D. NO. 19

NH$_2$-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

D hPTH 26-37 SEQ. I.D. NO. 20

NH$_2$-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

D hPTH 27-37 SEQ. I.D. NO. 21

NH$_2$-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

D hPTH 28-37 SEQ. I.D. NO: 22

NH$_2$-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (22)

D hPTH 29-37 SEQ. I.D. NO. 23

NH$_2$-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

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D hPTH 30-37 SEQ I.D. NO.24
NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

D hPTH 31-37 SEQ. I.D. NO.25
NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

D hPTH 32-37 SEQ. ID. NO.26
NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

D hPTH 33-37 SEQ. I.D. NO 27
NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

D hPTH 24-36 SEQ. I.D. NO.28
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

D hPTH 24-35 SEQ. ID. NO.29
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

D hPTH 24-34 SEQ I.D. NO.30
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

D hPTH 24-33 SEQ. I.D. NO.31
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

D hPTH 24-32 SEQ. I.D. NO:32
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

D hPTH 24-31 SEQ. I.D. NO.33
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

D hPTH 24-29 SEQ. I.D NO.34
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (34)

D hPTH 24-28 SEQ. I.D. NO.35
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (35)

The indicated sequences represent essential features of the secondary structure in their primary structure, as can be demonstrated by supporting NMR data. One precondition to this end was a determination of the PTH(1-37) secondary structure in physiological solution.

The above-mentioned regions of conspicuous structure have good immunogenic activity. Antibodies are formed, binding to the first amino acids of the N-terminus. Deficiency of only two amino acids gives rise to a substantial loss in affinity. Because these amino acids are indispensable for the biological activity to arise, it is possible by using the peptides of the invention to obtain antibodies recognizing only hPTH and fragments thereof which are biologically active.

Furthermore, antibodies can be produced which detect the mid-region 9-15, as well as antibodies giving C-terminal binding in the region of the amino acids 30-37. According to the invention, it is therefore possible to produce antibodies against hPTH(1-37) regions which, according to theoretical calculations, do not exhibit immunogenic activity within the entire molecule. In addition, these regions are separated from each other by such a far distance that no steric hindrance is present which would prevent simultaneous binding of two antibodies.

In preferred embodiments, the peptides may be modified at the N-terminal end, in the side-chain and/or at the C-terminal end, namely, taking the form of acetylation, amidation, phosphorylation and/or glycosylation products.

Eventually, the peptides of the invention may also be bound to carrier proteins such as hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin, or mouse serum albumin etc.. Binding to the carrier proteins is preferably effected using carbodiimide or formaldehyde.

The peptides of the invention may be used in the preparation of a diagnostic agent. The diagnostic agent of the invention can be obtained using the per se known immunization of animals with at least one of the peptides according to the invention. Following immunization, an immunoglobulin fraction can be isolated from the immunized animals, which contains antibody fractions having an antibody titer against at least one of the peptides of the invention. The invention is also directed to the antibodies thus obtained. In addition to the complete antibodies consisting of F_{ab} and F_c, fragments thereof such as F_{ab} or fragments of the antibodies being idiotypes of peptide epitopes may also be used in an alternative embodiment.

The peptides according to the invention are suitable for preparing an agent for the diagnosis of biologically active hPTH(1-37).

Referring to the following examples, the invention will be described in more detail.

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Example 1

Solid-Phase Synthesis of Peptides

The method of the invention for synthesizing the peptides is based on the peptide synthesis using a solid support. Each of the C-terminal amino acids is bound to the support material in the presence of dicyclohexylcarbodiimide and dimethylaminopyridine. Wang resin or similar resins are used as support material for the syntheses.

The following derivatives of L-amino acids are used in the synthesis of the sequence, starting from the peptidyl resin as specified: a) hPTH(1-10): Fmoc-Asn(Trt)-Wang resin, Fmoc-His(Trt)-OH, Fmoc-Met-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Boc-Ser(tBu)-OH; b) hPTH(9-18): Fmoc-Met-Wang resin, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Boc-His(Trt)-OH; c) hPTH(24-37): Fmoc-Leu-Wang resin, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Asn(Trt)-OH, Fmoc-His(Trt)-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH.

The synthesis may be carried out by in situ activation using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or derivatives thereof, or benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) or derivatives thereof in the presence of diisopropylethylamine or N-methylmorpholine and 1-hydroxybenzotriazole, using a four- to tenfold excess of Fmoc-L-amino acid during the coupling reactions in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. Removal of the Fmoc groups is effected using 20% piperidine

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or 2% piperidine and 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. Following synthesis, the resins are washed with 2-propanol and dichloromethane and dried to constant weight in a high vacuum.

Removal from the support and deprotection are carried out by reacting the peptidyl resin with trifluoroacetic acid containing 5% scavenger, water, ethanediol, phenol or thioanisole for 30-90 minutes at room temperature, filtrating, washing with trifluoroacetic acid, and subsequently precipitating with tert-butyl methyl ether. The precipitate is lyophilized from aqueous solution.

Example 2

Purification and Analysis

The raw products are purified by chromatography on a C18 reversed phase column (10 µm, buffer A: 0.01 N HCl in water; buffer B: 20% isopropanol, 30% methanol, 50% water, 0.01 N HCl; gradient: 10-80% within 60 minutes; detection at 230 nm).

The purity of the products is determined using mass spectrometry and C18 reversed phase chromatography.

Example 3

Coupling to Carrier Protein

Used as carrier proteins are hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin, or mouse serum albumin. Coupling is performed according to the carbodiimide method by way of the carboxyl groups of the peptides. The peptide is

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activated in aqueous solution by reaction with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride for 5 minutes. Coupling is effected by adding the activated peptide to an aqueous solution of the carrier. The molar ratio is 1 peptide on 50 amino acids of the carrier protein. The reaction takes 4 hours.

Page 11 is missing

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CLAIMS

1. The peptides from hPTH(1-37) having the sequence

hPTH 1-10

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18

NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18

NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18

NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18

NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18

NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18

NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 9-17

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

hPTH 9-16

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

hPTH 9-15

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

hPTH 9-14

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

hPTH 9-13

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

hPTH 24-37

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

hPTH 25-37

NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

hPTH 26-37

NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

hPTH 27-37

NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

hPTH 28-37

NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (22)

hPTH 29-37

NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

hPTH 30-37

NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

hPTH 31-37

NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

hPTH 32-37

NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

hPTH 33-37

NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

hPTH 24-36

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

hPTH 24-35

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

hPTH 24-34

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

hPTH 24-33

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

hPTH 24-32

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

hPTH 24-31

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

hPTH 24-29

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (34)

hPTH 24-28

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (35)

2. The peptides according to claim 1, which are modified at the N-terminal end, in the side-chain and/or at the C-terminal end, taking the form of acetylation, amidation, phosphorylation and/or glycosylation products and/or are bound to carrier proteins such as hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin, or mouse serum albumin.

3. A diagnostic agent which can be obtained using the _per se_ known immunization of animals with at least one of the peptides according to claim 1, recovering fractions containing immunoglobulins from the immunized animals, and isolating fractions having an antibody titer against at least one of the peptides according to claim 1, and which optionally contains additional adjuvants and/or vehicles.

4. Antibodies or fragments of antibodies, which can be obtained by immunizing animals with at least one of the peptides according to claim 1.

5. Use of the peptides according to claim 1 for producing an agent for the diagnosis of biologically active hPTH(1-37).

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Abstract

The present invention is directed to peptides from the sequence of hPTH(1-37), which contain α-helical amino acid sequence regions and/or non-structured amino acid sequence regions, said peptides being capable of inducing antibodies when injected into animals. Furthermore, the invention is directed to a diagnostic agent and antibodies obtainable by immunizing animals using the peptides according to the invention.

970208 US

DECLARATION AND POWER OF ATTORNEY

As a below named Inventor, I hereby declare that:

Attorney's Docket No. 07826-0007

My residence, post office address, and citizenship are as stated below next to my name. I believe I am an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: "PEPTIDES FROM THE DPTH (1-37) SEQUENCE", the specification of which

☐ is attached hereto.

☒ was filed on September 22, 1995 as PCT International Application No. PCT/EP95/03757 and was amended (if applicable) on ____________.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I do not know and do not believe that the same was ever known or used by others in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to the date of this application. I further state that the invention was not in public use or on sale in the United States of America more than one year prior to the date of this application. *I understand that I have a duty of candor and good faith toward the Patent and Trademark Office*, and I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate disclosing subject matter in common with the above-identified specification and having a filing date before that of the application on which priority is claimed:

Country	App. No.	Date of Filing	Priority Claimed Under 35 USC §119
German	DE 44 34 551.8	September 28, 1994	Yes ___x___ No_______

☐ I hereby claim the benefit under Title 35, United States Code, § 120 of any prior United States application(s), or §365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each claim of the present application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Filing Date	Status: patented, pending, abandoned

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

I hereby authorize the U.S. attorneys named herein to accept and follow instructions from Dr. Thomas Godemeyer, as to any action to be taken in the Patent and Trademark Office regarding this application, without direct communication between the U.S. attorney and the undersigned. In the event of a change in the persons from whom instructions may be taken, the U.S. attorney named herein will be notified by the undersigned.

POWER OF ATTORNEY: The following attorneys are hereby appointed to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Anthony B. Askew - 24,154; Roger T. Frost - 22,176; Jeffrey R. Young - 28,490; Robert R. Richards - 29,105; John R. Harris - 30,388; Stephen M. Schaetzel - 31,418; Larry A. Roberts - 31,871; Thomas A. Hodge - 22,602; Charles L. Warner II - 32,320; Gregory T. Gronholm - 32,415; Dale Lischer - 28,434; Peter G. Pappas - 33,205; James Dean Johnson - 31,771; Nora M. Tocups - 35,717; W. Scott Petty - 35,645; Daniel J. Warren - 34,272; Larry W. Shultz - 34,025; Hubert J. Barnhardt III - 36,729; Virginia L. Carron - 37,110; Leona G. Young - 37,265; Jamie L. Greene - 32,467; William A. Hartselle - 36,[illegible]; Holmes J. Hawkins III - 38,913; Mary Anthony Merchant - 39,771; Michael J. Mehrman - 40,086; William L. Warren - 36,714; Philip J. Parley - 38,445; P. Leslie Bessenger III - 39,108; James A. Witherspoon - 36,723; Brenda M. Drake - 40,339; James D. Withers - 40,376.

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Title: PEPTIDES FROM THE hPTH (1-37) SEQUENCE
Page 2

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Exhibit E

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Prüfungsantrag gem. § 44 PatG ist gestellt

(54) Peptide aus der Sequenz des hPTH (1-37)

(57) Gegenstand der vorliegenden Erfindung sind Peptide aus der Sequenz des hPTH (1-37), enthaltend α-helicale Aminosäuresequenzbereiche und/oder nicht strukturierte Aminosäuresequenzbereiche, wobei die Peptide bei Injektion in Tiere Antikörper zu induzieren vermögen. Ein weiterer Gegenstand der Erfindung sind ein Diagnostikum und Antikörper erhältlich durch Immunisierung von Tieren mit den erfindungsgemäßen Peptiden.

DE 44 34 551 A 1

Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen
BUNDESDRUCKEREI 02. 96 602 014/30

Beschreibung

Die vorliegende Erfindung betrifft Peptide aus der Sequenz des hPTH (1-37), ein Diagnostikum erhältlich durch Immunisierung von Tieren mit den Peptiden, Antikörper oder deren Fragmente erhältlich durch Immunisierung von Tieren mit den Peptiden sowie die Verwendung der Peptide zur Herstellung eines Mittels zur Diagnose von biologisch aktiven h-PTH.

Humanes Parathormon (hPTH), ein lineares Polypeptid aus 84 Aminosäuren, spielt eine wichtige Rolle in der Regulation des Calciumstoffwechsels. Der Metabolismus dieses Hormons führt zu einer großen Zahl C-terminaler Fragmente, deren biologische Funktion noch nicht geklärt ist. Als zirkulierendes N-terminales Fragment ist das hPTH 1-37 festgelegt (EP-A 0 349 545). Dieses Fragment besitzt die volle biologische Aktivität des Gesamthormons. Diese nimmt allerdings bei Verlust der ersten Aminosäure, Serin, deutlich ab und geht ohne die ersten beiden Aminosäuren, Serin und Valin, völlig verloren.

Für das intakte Hormon hPTH 1-84 und für N-terminales Fragment werden Serumkonzentrationen im Bereich von 10-12 mol/L gemessen. Zur Bestimmung solch niedriger Konzentrationen bedient man sich immunologischer Meßverfahren. Die validesten Ergebnisse liefern hierbei Meßverfahren nach dem Doppelantikörper oder Sandwich Prinzip (z. B. Two-site Radioimmunometric Assay, IRMA oder Sandwich Enzym Linked Immuno Sorbent Assay, Sandwich ELISA). Solche Assays sind für hPTH 1-84 kommerziell erhältlich. Zur Messung von hPTH 1-34 ist ein Assay nach dem Doppelantikörper-Prinzip nicht bekannt.

Hierfür sind zwei Antikörper notwendig. Diese müssen, um eine gegenseitige sterische Hinderung zu vermeiden, Epitope des Antigens erkennen, die in ausreichender Entfernung zueinander liegen. Bei Immunisierung mit dem intakten Antigen erhält man ein heterogenes Gemisch unterschiedlicher Antikörper, die für einen Sandwich-Assay erst aufwendig gereinigt werden müssen. Zwar war es bisher möglich aufgrund theoretischer Berechnungen nach B.A. Jameson & H. Wolf, The antigenic index: a novel algorithm for predicting antigenic determinants, CABIOS 4, p 181–186, 1988 am N-Terminus eine bevorzugte immunogen wirkende Sequenz im Bereich der Aminosäuren 7–14 festzustellen. Eine Immunisierung mit N-terminalen Fragmenten nach etablierten Methoden führt in erster Linie zu Antikörpern, die, wie für hPTH 1-34 beschrieben (J. Tampe, P. Brozio, H.E. Manneck, A. Mißbichler, E. Blind, K.B. Müller, H. Schmidt-Gayk und F.P. Armbruster; Characterisation of antibodies against human N-terminal parathyroid hormon by epitope mapping; J. Immunoassay 13 S. 1–13, 1992), in dem Bereich dieser Aminosäuren binden. Diese Antikörper können aber nicht zwischen biologisch aktiven und biologisch inaktiven PTH 1-84 oder Fragmenten davon, denen die ersten beiden Aminosäuren Serin und Valin fehlen, unterscheiden.

Das der Erfindung zugrunde liegende technische Problem besteht darin, Peptide anzugeben, mit deren Hilfe die oben genannten Nachteile der Diagnose von biologisch aktivem h-PTH beseitigt werden können.

Das angesprochene technische Problem wird überraschenderweise gelöst durch Peptide aus der Sequenz des hPTH (1-37) enthaltend α-helicale Aminosäuresequenzbereiche und/oder nicht strukturierte Aminosäuresequenzbereiche, wobei die Peptide bei Injektion in Tiere Antikörper zu induzieren vermögen. Dabei enthalten die Peptide vorzugsweise die N-terminale α-Helix im Bereich der Aminosäuren 5–9, einen unstrukturierten Abschnitt der Aminosäuren 10–16 und/oder eine C-terminale α-Helix im Bereich der Aminosäuresequenz 17–34 des hPTH (1-37). Vorzugsweise werden die folgenden erfindungsgemäßen Peptide zur Immunisierung verwendet:

hPTH 1-10 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH$ (1)
hPTH 1-9 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^5-His^9-OH$ (2)
hPTH 1-8 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH$ (3)
hPTH 1-7 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH$ (4)
hPTH 1-6 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH$ (5)
hPTH 1-5 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH$ (6)
hPTH 9-18 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{15}-OH$ (7)
hPTH 10-18 $NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{15}-OH$ (8)
hPTH 11-18 $NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (9)
hPTH 12-18 $NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{15}-OH$ (10)
hPTH 13-18 $NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (11)
hPTH 14-18 $NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{15}-OH$ (12)
hPTH 9-17 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH$ (13)
hPTH 9-16 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH$ (14)
hPTH 9-15 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH$ (15)
hPTH 9-14 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH$ (16)
hPTH 9-13 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH$ (17)
hPTH 24-37
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (18)
hPTH 25-37
$NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (19)
hPTH 26-37
$NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (20)
hPTH 27-37
$NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (21)

hPTH 28-37 $NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (22)
hPTH 29-37 $NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (23)
hPTH 30-37 $NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (24)
hPTH 31-37 $NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (25)
hPTH 32-37 $NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (26)
hPTH 33-37 $NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (27)
hPTH 24-36
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH$ (28)
hPTH 24-35
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH$ (29)
hPTH 24-34
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH$ (30)
hPTH 24-33 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}OH$ (31)
hPTH 24-32 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH$ (32)
hPTH 24-31 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH$ (33)
hPTH 24-30 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-OH$ (34)
hPTH 24-29 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH$ (35)
hPTH 24-28 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH$ (36)

Die genannten Sequenzen repräsentieren in ihrer Primärstruktur wesentliche Merkmale der Sekundärstruktur, wie sich durch NMR-Daten unterstützend belegen läßt. Voraussetzung dazu war eine Festlegung der Sekundärstruktur für PTH 1-37 in physiologischer Lösung.

Die genannten strukturell auffälligen Bereiche wirken gut immunogen. Es werden Antikörper gebildet, die an den ersten Aminosäuren des N-Terminus binden. Bereits das Fehlen von zwei Aminosäuren führt zu einem erheblichen Affinitätsverlust. Da diese Aminosäuren zur Ausübung der biologischen Wirkung unerläßlich sind, ist es mit dem erfindungsgemäßen Peptid möglich Antikörper zu erhalten, die nur hPTH und Fragmente davon erkennen, die biologisch aktiv sind.

Weiterhin sind Antikörper herstellbar, die den midregionalen Bereich 9—15 detektieren, und Antikörper die L-terminal im Bereich der Aminosäuren 30—37 binden. Erfindungsgemäß können also Antikörper gegen Bereiche des hPTH 1-37 produziert werden, die aufgrund theoretischer Berechnungen im Gesamtmolekül nicht immunogen wirken. Diese Bereiche liegen zudem soweit auseinander, daß keine sterische Hinderung vorliegt, die ein gleichzeitiges Binden zweier Antikörper verhindern würde.

Die Peptide können in bevorzugten Ausführungsformen am N-terminalen Ende, in der Seitenkette und/oder am C-terminalen Ende modifiziert sein, und zwar in Form von Acetylierungs-, Amidierungs-, Phosphorylierungs- und/oder Glycosylierungsprodukten.

Schließlich können erfindungsgemäße Peptide auch an Carrierproteine wie Hämocyanin, Thyroglobulin, Rinderserumalbumin, Ovalalbumin oder Mausserumalbumin etc. gebunden sein. Die Bindung an die Carrierproteine erfolgt vorzugsweise durch Carbodiimid oder Formaldehyd.

Die erfindungsgemäßen Peptide können verwendet werden, um ein Diagnostikum herzustellen. Das erfindungsgemäße Diagnostikum ist dabei erhältlich durch an sich bekannte Immunisierung von Tieren mit mindestens einem der erfindungsgemäßen Peptide. Nach der Immunisierung kann aus den immunisierten Tieren eine Immunoglobulin-Fraktion isoliert werden, die Antikörper-Fraktionen enthält, welche einen Antikörper-Titer gegen mindestens eines der erfindungsgemäßen Peptide aufweisen. Die so erhaltenen Antikörpern sind ebenfalls Gegenstand der vorliegenden Erfindung. In einer alternativen Ausführungsform können neben den vollständigen Antikörpern bestehend aus F_{ab} und F_c auch deren Fragmente wie F_{ab} oder Fragmente der Antikörper verwendet werden, welche die Idiotypen zu den Epitopen der Peptide sind.

Die Peptide gemäß der Erfindung sind zur Herstellung eines Mittels zur Diagnose von biologisch aktiven h-PTH (1-37) geeignet.

Die Erfindung wird anhand der folgenden Beispiele näher beschrieben:

Beispiel 1

Festphasensynthese der Peptide

Das erfindungsgemäße Verfahren zur Synthese der Peptide beruht auf der Peptidsynthese am festen Träger. Die C-terminale Aminosäure wird jeweils in Gegenwart von Dicyclohexylcarbodiimid und Dimethylaminopyridin an das Trägermaterial gebunden. Als Trägermaterial für die Synthesen werden Wang-Harz oder entsprechende Harze eingesetzt.

Folgende L-Aminosäure-Derivate werden für die Synthese der Sequenz, ausgehend vom aufgeführten Peptidyl-Harz, verwendet: a) hPTH 1-10: Fmoc—Asn(Trt)-Wang-Harz, Fmoc—His(Trt)—OH, Fmoc—Met—OH, Fmoc—Leu—OH, Fmoc—Gln(Trt)—OH, Fmoc—Ile—OH, Fmoc—Glu(OtBu)-OH, Fmoc—Ser(tBu)-OH, Fmoc—Val—OH, Bocer(tBu)-OH. b) hPTH 9-18: Fmoc—Met-Wang-Harz, Fmoc—Ser(tBu)-OH, Fmoc—Asn(Trt)—OH, Fmoc—Leu—OH, Fmoc—His(Trt)—OH, Fmoc—Lys(Boc)-OH, Fmoc—Gly—OH, Fmoc—Leu—OH, Fmoc—Asn(Trt)—OH, Boc-His(Trt)—OH. c) hPTH 24-37: Fmoc—Leu-Wang-Harz, Fmoc—Ala—OH, Fmoc—Val—OH, Fmoc—Phe—OH, Fmoc—Asn(Trt)—OH, Fmoc—His(Trt)—OH, Fmoc—Val—OH, Fmoc—Asp(OtBu)-OH, Fmoc—Gln(Trt)—OH, Fmoc—Leu—OH, Fmoc—Lys(Boc)-OH, Fmoc—Lys(Boc)-OH, Fmoc—Arg(Pmc)-OH, Fmoc—Leu—OH.

Die Synthese kann durch in situ-Aktivierung mit 2-(1H-Benzotriazol-1-yl)-1,1,3,3,-tetramethyluroniumtetrafluoroborat (TBTU) oder dessen Derivaten oder mit Benzotriazol-1-yl-oxytris-(dimethylamino)-phosphoniumhexafluorophosphat (BOP) der dessen Derivaten in Gegenwart von Diisopropylethylamin oder N-Methylmorpholin und 1-Hydroxybenzotriazol durchgeführt werden, wobei während der Kupplungen in N,N-Dimethylformamid, N,N-Dimethylacetamid oder N-Methylpyrrolidon ein vier- bis zehnfacher Überschuß der Fmoc—L-Aminosäure verwendet wird. Die Abspaltungen der Fmoc-Gruppen werden mit 20% Piperidin oder 2% Piperidin und 2% 1,8-Diazbicyclo[5,4,0]undec-7-en (DBU) in N,N-Dimethylformamid, N,N-Dimethylacetamid oder N-Methylpyrrolidon durchgeführt. Nach der Synthese werden die Harze mit 2-Propanol und Dichlormethan gewaschen und im Hochvakuum bis zur Gewichtskonstanz getrocknet.

Zur Abspaltung vom Träger und Entblockierung wird das Peptidyl-Harz 30—90 Minuten bei Raumtemperatur mit Trifluoressigsäure, die 5% Scavenger, Wasser, Ethandiol, Phenol oder Thioanisol, enthält, umgesetzt, filtriert, mit Trifluoressigsäure gewaschen und anschließend mit tert-Butylmethylether ausgefällt. Der Niederschlag wird aus wäßriger Lösung lyophilisiert.

Beispiel 2

Reinigung und Analyse

Die Reinigung der Rohprodukte erfolgt chromatographisch über eine C18-Reverse-Phase-Säule (10 µm, Puffer A: 0,01 N HCl in Wasser; Puffer B: 20% Isopropanol, 30% Methanol, 50% Wasser, 0,01 N HCl; Gradient: 10—80% in 60 Minuten; Detektion 230 nm).

Reinheit der Produkte werden durch Massenspektrometrie und C18-Reverse-Phase-Chromatographie bestimmt.

Beispiel 3

Kopplung an Carrierprotein

Als Carrierprotein werden Hämocyanin, Thyroglobulin, Rinderserumalbumin, Ovalbumin oder Mausserumalbumin verwendet. Die Kopplung erfolgt nach der Carbodiimid Methode über Carboxylgruppen des Peptides. Das Peptid wird in wäßriger Lösung durch 5 minütige Umsetzung mit 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimid Hydrochlorid aktiviert. Die Kopplung erfolgt durch Zugabe des aktivierten Peptides zu einer wäßrigen Lösung des Carriers. Das molare Verhältnis beträgt 1 Peptid auf 50 Aminosäuren des Carrierproteins. Die Umsetzung dauert 4 Stunden.

Die Reaktion wird durch Zugabe von Natriumacetat in einer Endkonzentration von 100 mM gestoppt. Man läßt eine Stunde inkubieren.

Die Abtrennung des Protein-Peptid Konjugates vom Peptid erfolgt durch wiederholte Dialyse gegen 100 mM Phosphatpuffer pH 7,2.

Beispiel 4

Synthese der Multiple Antigenic Pepides (MAP)

Die dreifache Lysin-Verzweigung wird erreicht, indem an C-terminales Alanin, gebunden an Wang-Harz, in drei Kupplungszyklen jeweils Fmoc—L-Lysin(Fmoc)—OH gebunden wird. Durch Abspaltung mit Piperidin werden danach acht freie Aminofunktionen erhalten, an denen die Sequenzen des humanen Parathormons nach obiger Beschreibung synthetisiert werden.

Beispiel 5

Immunisierung

Für die Erstimmunisierung werden pro kg Körpergewicht des zu immunisierenden Tieres 125 µg des Carrier-Peptid Konjugates bzw. MAP in 250 ml Wasser gelöst und mit 250 µl kompletten Freund'schen Adjuvans emulgiert. Die Emulsion wird über den Rücken verteilt in 10 Portionen s.c. appliziert.

Das Boostern erfolgt nach 2—4 Wochen analog. Hierbei wird lediglich das komplette Freund'sche Adjuvans durch inkomplettes Freund'sches Adjuvans ersetzt.

Patentansprüche

1. Peptide aus der Sequenz des hPTH (1-37) enthaltend α-helicale Aminosäuresequenzbereiche und/oder nicht strukturierte Aminosäuresequenzbereiche, wobei die Peptide bei Injektion in Tiere Antikörper zu induzieren vermögen.

2. Peptide nach Anspruch 1 aus hPTH (1-37) mit der Sequenz

hPTH 1-10 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH$ (1)
hPTH 1-9 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^5-His^9-OH$ (2)
hPTH 1-8 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^5-OH$ (3)

hPTH 1-7 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH$ (4)
hPTH 1-6 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH$ (5)
hPTH 1-5 $NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH$ (6)
hPTH 9-18 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{15}-OH$ (7)
hPTH 10-18 $NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (8)
hPTH 11-18 $NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{15}-OH$ (9)
hPTH 12-18 $NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{15}-OH$ (10)
hPTH 13-18 $NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{15}-OH$ (11)
hPTH 14-18 $NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH$ (12)
hPTH 9-17 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH$ (13)
hPTH 9-16 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-A_5n^{16}-OH$ (14)
hPTH 9-15 $NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH$ (15)
hPTH 9-14 $NH_2-His^9-Asn-^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH$ (16)
hPTH 9-13 $TH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH$ (17)
hPTH 24-37
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (18)
hPTH 25-37
$NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (19)
hPTH 26-37
$NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (20)
hPTH 27-37
$NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (21)
hPTH 28-37
$NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (22)
hPTH 29-37 $NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (23)
hPTH 30-37 $NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (24)
hPTH 31-37 $NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (25)
hPTH 32-37 $NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (26)
hPTH 33-37 $NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH$ (27)
hPTH 24-36
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH$ (28)
hPTH 24-35
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH$ (29)
hPTH 24-34
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH$ (30)
hPTH 24-33
$NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH$ (31)
hPTH 24-32 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH$ (32)
hPTH 24-31 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH$ (33)
hPTH 24-30 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-OH$ (34)
hPTH 24-29 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH$ (35)
hPTH 24-28 $NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH$ (36).

3. Peptide nach Anspruch 1 und/oder 2, die am N-terminalen Ende, in der Seitenkette und/oder am C-terminalen Ende modifiziert sind in Form von Acetylierungs-, Amidierungs-Phosphorylierungs- und/oder Glycosylierungsprodukten, und/oder gebunden sind an Carrierproteine wie Hämocyanin, Thyroglobulin, Rinderserumalbumin, Ovalalbumin oder Mausserumalbumin.
4. Diagnostikum, erhältlich durch an sich bekannte Immunisierung von Tieren mit mindestens einem der Peptide gemäß mindestens einem der Ansprüche 1 bis 3, Gewinnung von Immunoglobulinen enthaltenden Fraktionen aus den immunisierten Tieren und Isolierung von Fraktionen, die einen Antikörper-Titer gegen mindestens eines der Peptide gemäß mindestens einem der Ansprüche 1 bis 3 aufweisen und das gegebenenfalls weiter Hilfs- und/oder Trägerstoffe enthält.
5. Antikörper oder Fragmente von Antikörpern erhältlich durch Immunisierung von Tieren mit mindestens einem Peptid nach mindestens einem der Ansprüche 1 bis 3.
6. Verwendung der Peptide gemäß mindestens einem der Ansprüche 1 bis 3 zur Herstellung eines Mittels zur Diagnose von biologisch aktiven h-PTH (1-37).

- Leerseite -

Exhibit A

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(54) Title: PEPTIDES FROM THE hPTH SEQUENCE (1-37)

(54) Bezeichnung: PEPTIDE AUS DER SEQUENZ DES hPTH (1-37)

(57) Abstract

The invention concerns peptides from the human parathyroid hormone (hPTH) sequence (1-37) and containing α-helical amino acid sequence regions and/or non-structured amino acid sequence regions. The said peptides are capable of inducing antibodies when injected into animals. The invention also concerns a diagnostic agent and antibodies obtainable by vaccination of animals with the peptides in question.

(57) Zusammenfassung

Gegenstand der vorliegenden Erfindung sind Peptide aus der Sequenz des hPTH (1-37) enthaltend α-helicale Aminosäuresequenzbereiche und/oder nicht strukturierte Aminosäuresequenzbereiche, wobei die Peptide bei Injektion in Tiere Antikörper zu induzieren vermögen. Ein weiterer Gegenstand der Erfindung sind ein Diagnostikum und Antikörper erhältlich durch Immunisierung von Tieren mit den erfindungsgemäßen Peptiden.

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DE	Deutschland
DK	Dänemark
ES	Spanien
FI	Finnland
FR	Frankreich
GA	Gabon
GB	Vereinigtes Königreich
GE	Georgien
GN	Guinea
GR	Griechenland
HU	Ungarn
IE	Irland
IT	Italien
JP	Japan
KE	Kenya
KG	Kirgisistan
KP	Demokratische Volksrepublik Korea
KR	Republik Korea
KZ	Kasachstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxemburg
LV	Lettland
MC	Monaco
MD	Republik Moldau
MG	Madagaskar
ML	Mali
MN	Mongolei
MR	Mauretanien
MW	Malawi
NE	Niger
NL	Niederlande
NO	Norwegen
NZ	Neuseeland
PL	Polen
PT	Portugal
RO	Rumänien
RU	Russische Föderation
SD	Sudan
SE	Schweden
SI	Slowenien
SK	Slowakei
SN	Senegal
TD	Tschad
TG	Togo
TJ	Tadschikistan
TT	Trinidad und Tobago
UA	Ukraine
US	Vereinigte Staaten von Amerika
UZ	Usbekistan
VN	Vietnam

Peptide aus der Sequenz des hPTH (1-37)

Die vorliegende Erfindung betrifft Peptide aus der Sequenz des hPTH (1-37), ein Diagnostikum erhältlich durch Immunisierung von Tieren mit den Peptiden, Antikörper oder deren Fragmente erhältlich durch Immunisierung von Tieren mit den Peptiden sowie die Verwendung der Peptide zur Herstellung eines Mittels zur Diagnose von biologisch aktivem h-PTH.

Humanes Parathormon (hPTH), ein lineares Polypeptid aus 84 Aminosäuren, spielt eine wichtige Rolle in der Regulation des Calciumstoffwechsels. Der Metabolismus dieses Hormons führt zu einer großen Zahl C-terminaler Fragmente, deren biologische Funktion noch nicht geklärt ist. Als zirkulierendes N-terminales Fragment ist das hPTH 1-37 festgelegt (EP-A 0 349 545). Dieses Fragment besitzt die volle biologische Aktivität des Gesamthormons. Diese nimmt allerdings bei Verlust der ersten Aminosäure, Serin, deutlich ab und geht ohne die ersten beiden Aminosäuren, Serin und Valin, völlig verloren.

Für das intakte Hormon hPTH 1-84 und für das N-terminale Fragment werden Serumkonzentrationen im Bereich von 10^{-12} mol/l gemessen. Zur Bestimmung solch niedriger Konzentrationen bedient man sich immunologischer Meßverfahren. Die validesten Ergebnisse liefern hierbei Meßverfahren nach dem Doppelantikörper oder Sandwich Prinzip (z.B. Two-site Radioimmunometric Assay, IRMA oder Sandwich Enzym Linked Immuno Sorbent Assay, Sandwich ELISA). Solche Assays sind für hPTH 1-84 kommerziell erhältlich. Zur Messung von hPTH 1-34 ist ein Assay nach dem Doppelantikörper-Prinzip nicht bekannt.

Hierfür sind zwei Antikörper notwendig. Diese müssen, um eine gegenseitige sterische Hinderung zu vermeiden, Epitope des Antigens erkennen, die in ausreichender Entfernung zueinander liegen. Bei Immunisierung mit dem intakten Antigen erhält man ein heterogenes Gemisch unterschiedlicher Anti-

körper, die für einen Sandwich-Assay erst aufwendig gereinigt werden müssen. Zwar war es bisher möglich aufgrund theoretischer Berechnungen nach B.A. Jameson & H. Wolf, The antigenic index: a novel algorithm for predicting antigenic determinants, CABIOS 4, p 181-186, 1988 am N-Terminus eine bevorzugte immunogen wirkende Sequenz im Bereich der Aminosäuren 7 - 14 festzustellen. Eine Immunisierung mit N-terminalen Fragmenten nach etablierten Methoden führt in erster Linie zu Antikörpern, die, wie für hPTH 1-34 beschrieben (J. Tampe, P. Brozio, H.E. Manneck, A. Mißbichler, E. Blind, K.B. Müller, H. Schmidt-Gayk und F.P. Armbruster; Characterisation of antibodies against human N-terminal parathyroid hormon by epitope mapping; J. Immunoassay 13 S. 1-13, 1992), in dem Bereich dieser Aminosäuren binden. Diese Antikörper können aber nicht zwischen biologisch aktiven und biologisch inaktiven PTH 1-84 oder Fragmenten davon, denen die ersten beiden Aminosäuren Serin und Valin fehlen, unterscheiden.

Das der Erfindung zugrunde liegende technische Problem besteht darin, Peptide anzugeben, mit deren Hilfe die oben genannten Nachteile der Diagnose von biologisch aktivem h-PTH beseitigt werden können.

Das angesprochene technische Problem wird überraschenderweise gelöst durch Peptide aus der Sequenz des hPTH (1-37) enthaltend α-helicale Aminosäuresequenzbereiche und/oder nicht strukturierte Aminosäuresequenzbereiche, wobei die Peptide bei Injektion in Tiere Antikörper zu induzieren vermögen. Dabei enthalten die Peptide vorzugsweise die N-terminale α-Helix im Bereich der Aminosäuren 5-9, einen unstrukturierten Abschnitt der Aminosäuren 10-16 und/oder eine C-terminale α-Helix im Bereich der Aminosäuresequenz 17-34 des hPTH (1-37). Vorzugsweise werden die folgenden erfindungsgemäßen Peptide zur Immunisierung verwendet:

hPTH 1-10

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5

NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18

NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18

NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18

NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18

NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18

NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18

NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 9-17
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

hPTH 9-16
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

hPTH 9-15
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

hPTH 9-14
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

hPTH 9-13
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

hPTH 24-37
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

hPTH 25-37
NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

hPTH 26-37
NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

hPTH 27-37
NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

hPTH 28-37
NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (22)

hPTH 29-37
NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

hPTH 30-37

NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

hPTH 31-37

NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

hPTH 32-37

NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

hPTH 33-37

NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

hPTH 24-36

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

hPTH 24-35

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

hPTH 24-34

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

hPTH 24-33

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

hPTH 24-32

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

hPTH 24-31

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

hPTH 24-30

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-OH (34)

hPTH 24-29

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (35)

hPTH 24-28

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (36)

Die genannten Sequenzen repräsentieren in ihrer Primärstruktur wesentliche Merkmale der Sekundärstruktur, wie sich durch NMR-Daten unterstützend belegen läßt. Voraussetzung dazu war eine Festlegung der Sekundärstruktur für PTH 1-37 in physiologischer Lösung.

Die genannten strukturell auffälligen Bereiche wirken gut immunogen. Es werden Antikörper gebildet, die an den ersten Aminosäuren des N-Terminus binden. Bereits das Fehlen von zwei Aminosäuren führt zu einem erheblichen Affinitätsverlust. Da diese Aminosäuren zur Ausübung der biologischen Wirkung unerläßlich sind, ist es mit dem erfindungsgemäßen Peptid möglich Antikörper zu erhalten, die nur hPTH und Fragmente davon erkennen, die biologisch aktiv sind.

Weiterhin sind Antikörper herstellbar, die den midregionalen Bereich 9-15 detektieren, und Antikörper, die C-terminal im Bereich der Aminosäuren 30-37 binden. Erfindungsgemäß können also Antikörper gegen Bereiche des hPTH 1-37 produziert werden, die aufgrund theoretischer Berechnungen im Gesamtmolekül nicht immunogen wirken. Diese Bereiche liegen zudem soweit auseinander, daß keine sterische Hinderung vorliegt, die ein gleichzeitiges Binden zweier Antikörper verhindern würde.

Die Peptide können in bevorzugten Ausführungsformen am N-terminalen Ende, in der Seitenkette und/oder am C-terminalen Ende modifiziert sein, und zwar in Form von Acetylierungs-, Amidierungs-, Phosphorylierungs- und/oder Glycosylierungsprodukten.

Schließlich können erfindungsgemäße Peptide auch an Carrierproteine wie Hämocyanin, Thyroglobulin, Rinderserumalbumin, Ovalalbumin oder Mausserumalbumin etc. gebunden sein. Die Bindung an die Carrierproteine erfolgt vorzugsweise durch Carbodiimid oder Formaldehyd.

Die erfindungsgemäßen Peptide können verwendet werden, um ein Diagnostikum herzustellen. Das erfindungsgemäße Diagnostikum ist dabei erhältlich durch an sich bekannte Immunisierung von Tieren mit mindestens einem der erfindungsgemässen Peptide. Nach der Immunisierung kann aus den immunisierten Tieren eine Immunoglobulin-Fraktion isoliert werden, die Antikörper-Fraktionen enthält, welche einen Antikörper-Titer gegen mindestens eines der erfindungsgemäßen Peptide aufweisen. Die so erhaltenen Antikörpern sind ebenfalls Gegenstand der vorliegenden Erfindung. In einer alternativen Ausführungsform können neben den vollständigen Antikörpern bestehend aus F_{ab} und F_c auch deren Fragmente wie F_{ab} oder Fragmente der Antikörper verwendet werden, welche die Idiotypen zu den Epitopen der Peptide sind.

Die Peptide gemäß der Erfindung sind zur Herstellung eines Mittels zur Diagnose von biologisch aktiven h-PTH (1-37) geeignet.

Die Erfindung wird anhand der folgenden Beispiele näher beschrieben:

Beispiel 1

<u>Festphasensynthese der Peptide:</u>

Das erfindungsgemäße Verfahren zur Synthese der Peptide beruht auf der Peptidsynthese am festen Träger. Die C-terminale Aminosäure wird jeweils in Gegenwart von Dicyclohexylcarbodiimid und Dimethylaminopyridin an das Trägermaterial gebunden. Als Trägermaterial für die Synthesen werden Wang-

Harz oder entsprechende Harze eingesetzt.

Folgende L-Aminosäure-Derivate werden für die Synthese der Sequenz, ausgehend vom aufgeführten Peptidyl-Harz, verwendet: a) hPTH 1-10: Fmoc-Asn(Trt)-Wang-Harz, Fmoc-His(Trt)-OH, Fmoc-Met-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Boc-Ser(tBu)-OH. b) hPTH 9-18: Fmoc-Met-Wang-Harz, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Boc-His(Trt)-OH. c) hPTH 24-37: Fmoc-Leu-Wang-Harz, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Asn(Trt)-OH, Fmoc-His(Trt)-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Leu-OH.

Die Synthese kann durch in situ-Aktivierung mit 2-(1H-Benzotriazol-1-yl)-1,1,3,3,-tetramethyluroniumtetrafluoroborat (TBTU) oder dessen Derivaten oder mit Benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphoniumhexafluorophosphat (BOP) oder dessen Derivaten in Gegenwart von Diisopropylethylamin oder N-Methylmorpholin und 1-Hydroxybenzotriazol durchgeführt werden, wobei während der Kupplungen in N,N-Dimethylformamid, N,N-Dimethylacetamid oder N-Methylpyrrolidon ein vier- bis zehnfacher Überschuß der Fmoc-L-Aminosäure verwendet wird. Die Abspaltungen der Fmoc-Gruppen werden mit 20% Piperidin oder 2% Piperidin und 2% 1,8-Diazbicyclo[5,4,0]undec-7-en (DBU) in N,N-Dimethylformamid, N,N-Dimethylacetamid oder N-Methylpyrrolidon durchgeführt. Nach der Synthese werden die Harze mit 2-Propanol und Dichlormethan gewaschen und im Hochvakuum bis zur Gewichtskonstanz getrocknet.

Zur Abspaltung vom Träger und Entblockierung wird das Peptidyl-Harz 30-90 Minuten bei Raumtemperatur mit Trifluoressigsäure, die 5% Scavenger, Wasser, Ethandiol, Phenol oder Thioanisol, enthält, umgesetzt, filtriert, mit Trifluoressigsäure gewaschen und anschließend mit tert-Butylmethylet-

her ausgefällt. Der Niederschlag wird aus wäßriger Lösung lyophilisiert.

Beispiel 2

Reinigung und Analyse

Die Reinigung der Rohprodukte erfolgt chromatographisch über eine C18-Reverse-Phase-Säule (10µm, Puffer A: 0,01 N HCl in Wasser; Puffer B: 20% Isopropanol, 30 % Methanol, 50% Wasser, 0,01 N HCl; Gradient: 10-80% in 60 Minuten; Detektion 230 nm).

Die Reinheit der Produkte wird durch Massenspektrometrie und C18-Reverse-Phase-Chromatographie bestimmt.

Beispiel 3

Kopplung an Carrierprotein

Als Carrierprotein werden Hämocyanin, Thyroglobulin, Rinderserumalbumin, Ovalbumin oder Mausserumalbumin verwendet. Die Kopplung erfolgt nach der Carbodiimid-Methode über Carboxylgruppen des Peptides. Das Peptid wird in wässriger Lösung durch 5 minütige Umsetzung mit 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimid-Hydrochlorid aktiviert. Die Kopplung erfolgt durch Zugabe des aktivierten Peptides zu einer wässrigen Lösung des Carriers. Das molare Verhälnis beträgt 1 Peptid auf 50 Aminosäuren des Carrierproteins. Die Umsetzung dauert 4 Stunden.
Die Reaktion wird durch Zugabe von Natriumacetat in einer Endkonzentration von 100 mM gestoppt. Man läßt eine Stunde inkubieren.

Die Abtrennung des Protein-Peptid Konjugates vom Peptid erfolgt durch wiederholte Dialyse gegen 100 mM Phosphatpuffer pH 7,2.

Beispiel 4

Synthese der Multiple Antigenic Peptides (MAP)

Die dreifache Lysin-Verzweigung wird erreicht, indem an C-terminales Alanin , gebunden an Wang-Harz, in drei Kupplungszyklen jeweils Fmoc-L-Lysin(Fmoc)-OH gebunden wird. Durch Abspaltung mit Piperidin werden danach acht freie Aminofunktionen erhalten, an denen die Sequenzen des humanen Parathormons nach obiger Beschreibung synthetisiert werden.

Beispiel 5

Immunisierung

Für die Erstimmunisierung werden pro kg Körpergewicht des zu immunisierenden Tieres 125 µg des Carrier-Peptid Konjugates bzw. MAP in 250 ml Wasser gelöst und mit 250 µl kompletten Freund'schen Adjuvans emulgiert. Die Emulsion wird über den Rücken verteilt in 10 Portionen s.c. appliziert.

Das Boostern erfolgt nach 2-4 Wochen analog. Hierbei wird lediglich das komplette Freund'sche Adjuvans durch inkomplettes Freund'sches Adjuvans ersetzt.

Patentansprüche

1. Peptide aus der Sequenz des hPTH (1-37) enthaltend α-helicale Aminosäuresequenzbereiche und/oder nicht strukturierte Aminosäuresequenzbereiche, wobei die Peptide bei Injektion in Tiere Antikörper zu induzieren vermögen.

2. Peptide nach Anspruch 1 aus hPTH (1-37) mit der Sequenz

hPTH 1-10
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18
NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18

NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18

NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18

NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18

NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18

NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 9-17

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

hPTH 9-16

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

hPTH 9-15

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

hPTH 9-14

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

hPTH 9-13

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

hPTH 24-37

NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-
Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

hPTH 25-37
NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

hPTH 26-37
NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

hPTH 27-37
NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

hPTH 28-37
NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (22)

hPTH 29-37
NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

hPTH 30-37
NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

hPTH 31-37
NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

hPTH 32-37
NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

hPTH 33-37
NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

hPTH 24-36
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

hPTH 24-35
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

hPTH 24-34
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

hPTH 24-33
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

hPTH 24-32
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

hPTH 24-31
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

hPTH 24-30
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-OH (34)

hPTH 24-29
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (35)

hPTH 24-28
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (36)

3. Peptide nach Anspruch 1 und/oder 2, die am N-terminalen Ende, in der Seitenkette und/oder am C-terminalen Ende modifiziert sind in Form von Acetylierungs-, Amidierungs-, Phosphorylierungs- und/oder Glycosylierungsprodukten, und/oder gebunden sind an Carrierproteine wie Hämocyanin, Thyroglobulin, Rinderserumalbumin, Ovalalbumin oder Mausserumalbumin.

4. Diagnostikum, erhältlich durch an sich bekannte Immunisierung von Tieren mit mindestens einem der Peptide gemäß mindestens einem der Ansprüche 1 bis 3, Gewinnung von Immunoglobulinen enthaltenden Fraktionen aus den immunisierten Tieren und Isolierung von Fraktionen, die einen Antikörper-Titer gegen mindestens eines der Peptide gemäß mindestens einem der Ansprüche 1 bis 3 aufweisen und das gegebenenfalls weiter Hilfs- und/oder Trägerstoffe enthält.

5. Antikörper oder Fragmente von Antikörpern erhältlich durch Immunisierung von Tieren mit mindestens einem Peptid nach mindestens einem der Ansprüche 1 bis 3.

6. Verwendung der Peptide gemäß mindestens einem der Ansprüche 1 bis 3 zur Herstellung eines Mittels zur Diagnose von biologisch aktiven h-PTH (1-37).

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/03757

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/635 C07K16/24 G01N33/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 03201 (HILLIKER SANDRA R) 17 February 1994 see claims; example 1 ---	1,2
X	CHEMICAL ABSTRACTS, vol. 96, no. 21, 24 May 1982 Columbus, Ohio, US; abstract no. 174594, NAKAMURA, RYUICHI ET AL 'Action of fragments of human parathyroid hormone on blood pressure in rats' see abstract & ENDOCRINOL. JPN. (1981), 28(4), 547-9 CODEN: ECJPAE;ISSN: 0013-7219, 1981 --- -/--	1,2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
10 January 1996	26. 01. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Fuhr, C

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 96, no. 5, 1 February 1982 Columbus, Ohio, US; abstract no. 29060, NUSSBAUM, SAMUEL R. ET AL 'Monoclonal antibodies directed against the biologically active region of parathyroid hormone' see abstract & MONOCLONAL ANTIBODIES ENDOCR. RES. (1981), 181-92. EDITOR(S): FELLOWS, ROBERT E.;EISENBARTH, GEORGE S. PUBLISHER: RAVEN, NEW YORK, N. Y. CODEN: 46PAAZ, 1981 ---	1,2,4-6
X	JOURNAL OF IMMUNOASSAY, vol. 13, no. 1, 1992 NEW YORK, US, pages 1-13, J. TAMPE ET AL. 'Characterization of Antibodies against Human N-Terminal Parathyroid Hormone by Epitope Mapping' cited in the application see page 2, paragraph 2 see page 4, paragraph 1 see results and discussion of the pages 5-12; ---	1-6
X	J. PHARMACOL. EXP. THER. (1981), 216(3), 567-71 CODEN: JPETAB;ISSN: 0022-3565, 1981 PANG, PETER K. T. ET AL 'Hypotensive action of synthetic fragments of parathyroid hormone' see page 567, right column, paragraph 2 see page 568, right column, paragraph 5; figure 1; table 1 ---	1,2
X	CHEMICAL ABSTRACTS, vol. 95, no. 17, 26 October 1981 Columbus, Ohio, US; abstract no. 144316, HASHIMOTO, KEITARO ET AL 'Effects of parathyroid hormone and related polypeptides on the heart and coronary circulation of dogs' see abstract & J. CARDIOVASC. PHARMACOL. (1981), 3(4), 668-76 CODEN: JCPCDT;ISSN: 0160-2446, 1981 ---	1,2
X	FR,A,2 550 204 (TOYO JOZO KK) 8 February 1985 see fragment 10 of page 25 and fragment 40 of page 50. -/--	1-3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E. Wingender et al. 'Structure-Function Relationship in Parathyroid Hormone' in: Advances in Protein Design, International Workshop 1988, GBF Monographs, Vol. 12, ed. by H. Blöcker, J. Collins, R.D. Schmid, D. Schomburg; pub. by VCH, 1988, p. 167-176, see page 169, paragraph 3 ---	1
X	ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, vol. 208, 1986 NEW YORK, US, pages 315-327, L.H. CAPORALE AND M. ROSENBLATT 'Parathyroid Hormone Antagonists effective in vivo' see figure 1 ---	1
X	WO,A,91 06564 (FORSSMANN WOLF GEORG) 16 May 1991 see page 5, paragraph 3; claims 1-5,14 ---	1,3-6
A	DE,A,33 47 548 (JUEPPNER HARALD WERNER DR MED;HESCH ROLF DIETER) 11 July 1985 see claims; examples -----	1,4-6

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9403201	17-02-94	CA-A-	2141588	17-02-94
		EP-A-	0656784	14-06-95
FR-A-2550204	08-02-85	JP-C-	1684818	31-07-92
		JP-B-	3052479	12-08-91
		JP-A-	60034996	22-02-85
		JP-A-	61024598	03-02-86
		DE-A-	3428942	28-02-85
		US-A-	4656250	07-04-87
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		AU-B-	643725	25-11-93
		AU-B-	6871291	31-05-91
		CA-A-	2071538	28-04-91
		DE-D-	59008949	24-05-95
		EP-A-	0497915	12-08-92
		ES-T-	2071837	01-07-95
DE-A-3347548	11-07-85	NONE		

INTERNATIONALL .ECHERCHENBERICHT

Inter ... Aktenzeichen
PCT/EP 95/03757

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
IPK 6 C07K14/635 C07K16/24 G01N33/78

Nach der Internationalen Patentklassifikation (IPK) oder nach der nationalen Klassifikation und der IPK

B. RECHERCHIERTE GEBIETE

Recherchierter Mindestprüfstoff (Klassifikationssystem und Klassifikationssymbole)
IPK 6 C07K G01N

Recherchierte aber nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Gebiete fallen

Während der internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)

C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	WO,A,94 03201 (HILLIKER SANDRA R) 17.Februar 1994 siehe Ansprüche; Beispiel 1 ---	1,2
X	CHEMICAL ABSTRACTS, vol. 96, no. 21, 24.Mai 1982 Columbus, Ohio, US; abstract no. 174594, NAKAMURA, RYUICHI ET AL 'Action of fragments of human parathyroid hormone on blood pressure in rats' siehe Zusammenfassung & ENDOCRINOL. JPN. (1981), 28(4), 547-9 CODEN: ECJPAE;ISSN: 0013-7219, 1981 --- -/--	1,2

[X] Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

[X] Siehe Anhang Patentfamilie

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Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
10.Januar 1996	26.01.96

Name und Postanschrift der Internationale Recherchenbehörde	Bevollmächtigter Bediensteter
Europäisches Patentamt, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Fuhr, C

Formblatt PCT/ISA/210 (Blatt 2) (Juli 1992)

C.(Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	CHEMICAL ABSTRACTS, vol. 96, no. 5, 1.Februar 1982 Columbus, Ohio, US; abstract no. 29060, NUSSBAUM, SAMUEL R. ET AL 'Monoclonal antibodies directed against the biologically active region of parathyroid hormone' siehe Zusammenfassung & MONOCLONAL ANTIBODIES ENDOCR. RES. (1981), 181-92. EDITOR(S): FELLOWS, ROBERT E.;EISENBARTH, GEORGE S. PUBLISHER: RAVEN, NEW YORK, N. Y. CODEN: 46PAAZ, 1981 ---	1,2,4-6
X	JOURNAL OF IMMUNOASSAY, Bd. 13, Nr. 1, 1992 NEW YORK, US, Seiten 1-13, J. TAMPE ET AL. 'Characterization of Antibodies against Human N-Terminal Parathyroid Hormone by Epitope Mapping' in der Anmeldung erwähnt siehe Seite 2, Absatz 2 siehe Seite 4, Absatz 1 siehe 'results' und 'discussion' auf den Seiten 5-12 ---	1-6
X	J. PHARMACOL. EXP. THER. (1981), 216(3), 567-71 CODEN: JPETAB;ISSN: 0022-3565, 1981 PANG, PETER K. T. ET AL 'Hypotensive action of synthetic fragments of parathyroid hormone' siehe Seite 567, rechte Spalte, Absatz 2 siehe Seite 568, rechte Spalte, Absatz 5; Abbildung 1; Tabelle 1 ---	1,2
X	CHEMICAL ABSTRACTS, vol. 95, no. 17, 26.Oktober 1981 Columbus, Ohio, US; abstract no. 144316, HASHIMOTO, KEITARO ET AL 'Effects of parathyroid hormone and related polypeptides on the heart and coronary circulation of dogs' siehe Zusammenfassung & J. CARDIOVASC. PHARMACOL. (1981), 3(4), 668-76 CODEN: JCPCDT;ISSN: 0160-2446, 1981 ---	1,2
X	FR,A,2 550 204 (TOYO JOZO KK) 8.Februar 1985 siehe Fragment 10 auf Seite 25 und Fragment 40 auf Seite 50 --- -/--	1-3

C.(Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	E. Wingender et al. 'Structure-Function Relationship in Parathyroid Hormone' in: Advances in Protein Design, International Workshop 1988, GBF Monographs, Vol. 12, ed. by H. Blöcker, J. Collins, R.D. Schmid, D. Schomburg; pub. by VCH, 1988, p. 167-176, siehe Seite 169, Absatz 3	1
X	ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, Bd. 208, 1986 NEW YORK, US, Seiten 315-327, L.H. CAPORALE AND M. ROSENBLATT 'Parathyroid Hormone Antagonists effective in vivo' siehe Abbildung 1	1
X	WO,A,91 06564 (FORSSMANN WOLF GEORG) 16.Mai 1991 siehe Seite 5, Absatz 3; Ansprüche 1-5,14	1,3-6
A	DE,A,33 47 548 (JUEPPNER HARALD WERNER DR MED;HESCH ROLF DIETER) 11.Juli 1985 siehe Ansprüche; Beispiele	1,4-6

Im Recherchenbericht angeführtes Patentdokument	Datum der Veröffentlichung	Mitglied(er) der Patentfamilie		Datum der Veröffentlichung
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		EP-A-	0656784	14-06-95
FR-A-2550204	08-02-85	JP-C-	1684818	31-07-92
		JP-B-	3052479	12-08-91
		JP-A-	60034996	22-02-85
		JP-A-	61024598	03-02-86
		DE-A-	3428942	28-02-85
		US-A-	4656250	07-04-87
WO-A-9106564	16-05-91	DE-A-	3935738	08-05-91
		AT-T-	121424	15-05-95
		AU-B-	643725	25-11-93
		AU-B-	6871291	31-05-91
		CA-A-	2071538	28-04-91
		DE-D-	59008949	24-05-95
		EP-A-	0497915	12-08-92
		ES-T-	2071837	01-07-95
DE-A-3347548	11-07-85	KEINE		

Exhibit

1

US006030790A

United States Patent [19]

Adermann et al.

[11] Patent Number: 6,030,790

[45] Date of Patent: Feb. 29, 2000

[54] ANTIBODIES THAT BIND PEPTIDES FROM THE HPTH SEQUENCE (1-37)

[75] Inventors: Knut Adermann, Hannover; Dieter Hock, Neckarbischofsheim; Markus Mägerlein, Obernburg, all of Germany

[73] Assignee: Haemopep Pharma GmbH, Germany

[21] Appl. No.: 08/817,547

[22] PCT Filed: Sep. 22, 1995

[86] PCT No.: PCT/EP95/03757

§ 371 Date: Mar. 27, 1997

§ 102(e) Date: Mar. 27, 1997

[87] PCT Pub. No.: WO96/10041

PCT Pub. Date: Apr. 4, 1996

[30] Foreign Application Priority Data

Sep. 28, 1994 [DE] Germany ..... P 44 34 551

[51] Int. Cl.[7] G01N 33/43; C07K 16/26

[52] U.S. Cl. 435/7.1; 436/512; 530/387.1; 530/387.2; 530/387.9; 530/388.24

[58] Field of Search 435/7.1; 436/512; 530/387.1, 387.2, 387.9, 388.24

[56] References Cited

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Ngo et al. The Protein Folding Problem and Tertiary Structure Prediction, Merz et al., eds., Birkhauser, Boston, pp. 492–495, 1994.

Primary Examiner—Elizabeth Kemmerer

Attorney, Agent, or Firm—Jones & Askew, LLP

[57] ABSTRACT

The present invention is directed to peptides from the sequence of hPTH(1–37), which contain α-helical amino acid sequence regions and/or non-structured amino acid sequence regions, said peptides being capable of inducing antibodies when injected into animals. Furthermore, the invention is directed to a diagnostic agent and antibodies obtainable by immunizing animals using the peptides according to the invention.

25 Claims, No Drawings

ANTIBODIES THAT BIND PEPTIDES FROM THE HPTH SEQUENCE (1-37)

This application was filed under 35 U.S.C. § 371 and claims priority from PCT/EP95/03757, filed Sep. 22, 1995.

The present invention relates to peptides from the sequence of hPTH(1–37), and the use of said peptides in the preparation of an agent for diagnosing biologically active hPTH.

Human parathyroid hormone (hPTH), a linear polypeptide having 84 amino acids, plays an important role in the regulation of the calcium metabolism. The metabolism of this hormone gives rise to a large number of C-terminal fragments, the biological functions of which have not yet been elucidated. The hPTH(1–37) has been established as a circulating N-terminal fragment (EP-A 0 349 545). This fragment has the full biological activity of the entire hormone. However, upon loss of the first amino acid, serine, the activity significantly decreases and is lost completely without the first two amino acids, serine and valine.

Serum levels in the range of 10^{-12} mol/l are measured for the intact hormone hPTH(1–84) and for the N-terminal fragment. Immunological measuring procedures are employed to determine such low concentrations. Here, the most valid results are provided by measuring procedures according to the double antibody or sandwich principle (e.g., the two-site radioimmunometric assay, IRMA, or the sandwich enzyme-linked immuno sorbent assay, Sandwich ELISA). For hPTH(1–84), such assays are commercially available. For the measurement of hPTH(1–34), an assay according to the double antibody principle is not known.

Here, two antibodies are required. In order to avoid mutual steric hindrance, they must be capable of recognizing antigen epitopes located at a sufficient distance from each other. When immunizing using the intact antigen, a heterogeneous mixture of various antibodies is obtained, which first must be subjected to an expensive purification in order to conduct a sandwich assay. According to theoretical calculations by B. A. Jameson and H. Wolf, The Antigenic Index: A Novel Algorithm for Predicting Antigenic Determinants, CABIOS 4, p. 181–186, 1988; it has been possible so far to detect a preferred sequence having immunogenic activity in the region of the amino acids 7–14 at the N-terminus. Immunization with N-terminal fragments according to established methods predominantly results in antibodies which, as has been described for hPTH(1–34) (J. Tampe, P. Brozio, H. E. Manneck, A. Miβbichler, E. Blind, K. B. Millers, H. SchmidtGayk, and F. P. Armbruster, Characterisation of Antibodies Against Human N-Terminal Parathyroid Hormone by Epitope Mapping; J. Immunoassay 13, p. 1–13, 1992), bind in the region of these amino acids. However, these antibodies are not capable of discriminating between biologically active and biologically inactive PTH (1–84) or fragments thereof lacking the first two amino acids serine and valine.

The technical problem which this invention is based upon is to provide peptides by means of which it is possible to eliminate the above-mentioned drawbacks in the diagnosis of biologically active hPTH.

Surprisingly, the technical problem described above is solved by means of the following peptides from the sequence of hPTH(1–37):

hPTH 1-10 SEQ I.D. NO.1
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-Asn^{10}-OH (1)

hPTH 1-9 SEQ I.D. NO.2
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-His^9-OH (2)

hPTH 1-8 SEQ I.D. NO.3
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-Met^8-OH (3)

hPTH 1-7 SEQ I.D. NO.4
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-Leu^7-OH (4)

hPTH 1-6 SEQ I.D. NO.5
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-Gln^6-OH (5)

hPTH 1-5 SEQ I.D. NO.6
NH_2-Ser^1-Val^2-Ser^3-Glu^4-Ile^5-OH (6)

hPTH 9-18 SEQ I.D. NO.7
NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (7)

hPTH 10-18 SEQ I.D. NO.8
NH_2-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (8)

hPTH 11-18 SEQ I.D. NO.9
NH_2-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (9)

hPTH 12-18 SEQ I.D. NO.10
NH_2-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (10)

hPTH 13-18 SEQ I.D. NO.11
NH_2-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (11)

hPTH 14-18 SEQ I.D. NO.12
NH_2-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-Met^{18}-OH (12)

hPTH 9-17 SEQ I.D. NO.13
NH_2-His^9-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-Ser^{17}-OH (13)

hPTH 9-16 SEQ I.D. NO.14

-continued

NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-Asn^{16}-OH (14)

hPTH 9-15 SEQ I.D. NO.15
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-Leu^{15}-OH (15)

hPTH 9-14 SEQ I.D. NO.16
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-His^{14}-OH (16)

hPTH 9-13 SEQ I.D. NO.17
NH_2-His^{9}-Asn^{10}-Leu^{11}-Gly^{12}-Lys^{13}-OH (17)

hPTH 24-37 SEQ I.D. NO.18
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (18)

hPTH 25-37 SEQ I.D. NO.19
NH_2-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (19)

hPTH 26-37 SEQ I.D. NO.20
NH_2-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (20)

hPTH 27-37 SEQ I.D. NO.21
NH_2-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (21)

hPTH 28-37 SEQ I.D. NO.22
NH_2-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (22)

hPTH 29-37 SEQ I.D. NO.23
NH_2-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (23)

hPTH 30-37 SEQ I.D. NO.24
NH_2-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (24)

hPTH 31-37 SEQ I.D. NO.25
NH_2-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (25)

hPTH 32-37 SEQ I.D. NO.26
NH_2-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (26)

hPTH 33-37 SEQ I.D. NO.27
NH_2-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-Leu^{37}-OH (27)

hPTH 24-36 SEQ I.D. NO.28
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-Ala^{36}-OH (28)

hPTH 24-35 SEQ I.D. NO.29
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-Val^{35}-OH (29)

hPTH 24-34 SEQ I.D. NO.30
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-Phe^{34}-OH (30)

hPTH 24-33 SEQ I.D. NO.31
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-Asn^{33}-OH (31)

hPTH 24-32 SEQ I.D. NO.32
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-His^{32}-OH (32)

hPTH 24-31 SEQ I.D. NO.33
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-Asp^{30}-Val^{31}-OH (33)

hPTH 24-29 SEQ I.D. NO.34
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-Gln^{29}-OH (34)

hPTH 24-38 SEQ I.D. NO.35
NH_2-Leu^{24}-Arg^{25}-Lys^{26}-Lys^{27}-Leu^{28}-OH (35)

The indicated sequences represent essential features of the secondary structure in their primary structure, as can be demonstrated by supporting NMR data. One precondition to this end was a determination of the PTH(1–37) secondary structure in physiological solution.

The above-mentioned regions of conspicuous structure have good immunogenic activity. Antibodies are formed, binding to the first amino acids of the N-terminus. Deficiency of only two amino acids gives rise to a substantial loss in affinity. Because these amino acids are indispensable

for the biological activity to arise, it is possible by using the peptides of the invention to obtain antibodies recognizing only hPTH and fragments thereof which are biologically active.

Furthermore, antibodies can be produced which detect the mid-region 9–15, as well as antibodies giving C-terminal binding in the region of the amino acids 30–37. According to the invention, it is therefore possible to produce antibodies against hPTH(1–37) regions which, according to theoretical calculations, do not exhibit immunogenic activity within the entire molecule. In addition, these regions are separated from each other by such a far distance that no steric hindrance is present which would prevent simultaneous binding of two antibodies.

In preferred embodiments, the peptides may be modified at the N-terminal end, in the side-chain and/or at the C-terminal end, namely, taking the form of acetylation, amidation, phosphorylation and/or glycosylation products.

Eventually, the peptides of the invention may also be bound to carrier proteins such as hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin, or mouse serum albumin etc. Binding to the carrier proteins is preferably effected using carbodiimide or formaldehyde.

The peptides of the invention may be used in the preparation of a diagnostic agent. The diagnostic agent of the invention can be obtained using the per se known immunization of animals with at least one of the peptides according to the invention. Following immunization, an immunoglobulin fraction can be isolated from the immunized animals, which contains antibody fractions having an antibody titer against at least one of the peptides of the invention. The invention is also directed to the antibodies thus obtained. In addition to the complete antibodies consisting of F_{ab} and F_c, fragments thereof such as F_{ab} or fragments of the antibodies being idiotypes of peptide epitopes may also be used in an alternative embodiment.

The peptides according to the invention are suitable for preparing an agent for the diagnosis of biologically active hPTH(1–37).

Referring to the following examples, the invention will be described in more detail.

EXAMPLE 1

Solid-Phase Synthesis of Peptides

The method of the invention for synthesizing the peptides is based on the peptide synthesis using a solid support. Each of the C-terminal amino acids is bound to the support material in the presence of dicyclohexylcarbodiimide and dimethylaminopyridine. Wang resin or similar resins are used as support material for the syntheses.

The following derivatives of L-amino acids are used in the synthesis of the sequence, starting from the peptidyl resin as specified: a) hPTH(1–10) Seq. I.D. No. 1: Fmoc-Asn(Trt)-Wang resin, Fmoc-His(Trt)-OH, Fmoc-Met-OH, Fmoc-Leu-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Boc-Ser(tBu)-OH; b) hPTH(9–18) Seq. I.D. No. 7): Fmoc-Met-Wang resin, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Leu-OH, Fmoc-His(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Boc-His(Trt)-OH; c) hPTH(24–37) Seq. I.D. No. 18: Fmoc-Leu-Wang resin, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Asn(Trt)-OH, Fmoc-His(Trt)-OH, Fmoc-Val-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Arg (Pmc)-OH, Fmoc-Leu-OH.

The synthesis may be carried out by in situ activation using 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or derivatives thereof, or benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) or derivatives thereof in the presence of diisopropylethylamine or N-methylmorpholine and 1-hydroxybenzotriazole, using a four- to tenfold excess of Fmoc-L-amino acid during the coupling reactions in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. Removal of the Fmoc groups is effected using 20% piperidine or 2% piperidine and 2% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in N,N-dimethylformamide, N,N-dimethylacetamide or N-methylpyrrolidone. Following synthesis, the resins are washed with 2-propanol and dichloromethane and dried to constant weight in a high vacuum.

Removal from the support and deprotection are carried out by reacting the peptidyl resin with trifluoroacetic acid containing 5% scavenger, water, ethanediol, phenol or thioanisole for 30–90 minutes at room temperature, filtrating, washing with trifluoroacetic acid, and subsequently precipitating with tert-butyl methyl ether. The precipitate is lyophilized from aqueous solution.

EXAMPLE 2

Purification and Analysis

The raw products are purified by chromatography on a C18 reversed phase column (10 μm, buffer A: 0.01 N HCl in water; buffer B: 20% isopropanol, 30% methanol, 50% water, 0.01 N HCl; gradient: 10–80% within 60 minutes; detection at 230 nm).

The purity of the products is determined using mass spectrometry and C18 reversed phase chromatography.

EXAMPLE 3

Coupling to Carrier Protein

Used as carrier proteins are hemocyanin, thyroglobulin, bovine serum albumin, ovalbumin, or mouse serum albumin. Coupling is performed according to the carbodiimide method by way of the carboxyl groups of the peptides. The peptide is activated in aqueous solution by reaction with 1-ethyl-3-(3-methylaminopropyl)carbodiimide hydrochloride for 5 minutes. Coupling is effected by adding the activated peptide to an aqueous solution of the carrier. The molar ratio is 1 peptide 50 amino acids of the carrier protein. The reaction takes 4 hours.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 36

-continued

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ser Val Ser Glu Ile Gln Leu Met His Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ser Val Ser Glu Ile Gln Leu Met His
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ser Val Ser Glu Ile Gln Leu Met
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ser Val Ser Glu Ile Gln Leu
1 5

-continued

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ser Val Ser Glu Ile Gln
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Ser Val Ser Glu Ile
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

His Asn Leu Gly Lys His Leu Asn Ser Met
1 5 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Asn Leu Gly Lys His Leu Asn Ser Met
1 5

-continued

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Leu Gly Lys His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Gly Lys His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Lys His Leu Asn Ser Met
1 5

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

His Leu Asn Ser Met

-continued

1 5

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

His Asn Leu Gly Lys His Leu Asn Ser
1 5

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

His Asn Leu Gly Lys His Leu Asn
1 5

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

His Asn Leu Gly Lys His Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

-continued

His Asn Leu Gly Lys His
1 5

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

His Asn Leu Gly Lys
1 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

-continued

Lys Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Lys Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Leu Gln Asp Val His Asn Phe Val Ala Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Gln Asp Val His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Asp Val His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Val His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

His Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Asn Phe Val Ala Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe Val
1 5 10

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Leu Arg Lys Lys Leu Gln Asp Val His Asn Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Leu Arg Lys Lys Leu Gln Asp Val His Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

-continued

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Leu Arg Lys Lys Leu Gln Asp Val His
1 5

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Leu Arg Lys Lys Leu Gln Asp Val
1 5

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Leu Arg Lys Lys Leu Gln Asp
1 5

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Leu Arg Lys Lys Leu Gln
1 5

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

-continued

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Leu Arg Lys Lys Leu
1 5

We claim:

1. A kit for detecting active human parathyroid hormone (hPTH) comprising a container and a first group of antibodies or antibody fragments and a second group of antibodies or antibody fragments, wherein the first group selectively binds a peptide of hPTH selected from the group consisting of peptides having SEQ. ID. Nos. 1–6 and the second group selectively binds hPTH at an epitope contained within amino acids 24 to 37.

2. The kit of claim 1, wherein the second group of antibodies or antibody fragments selectively binds a peptide of hPTH selected from the group consisting of peptides having SEQ. ID. Nos. 18–36.

3. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 1.

4. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 2.

5. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 3.

6. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 4.

7. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 5.

8. The kit of claim 1, wherein the first group of antibodies or antibody fragments selectively bind peptides of hPTH having SEQ. ID. No. 6.

9. An immunological method of detecting active human parathyroid hormone (hPTH) in a sample comprising:

contacting the sample with a first antibody or antibody fragment which selectively binds a peptide of hPTH selected from the group consisting of peptides having SEQ. ID. Nos. 1–6, wherein the first antibody or antibody fragment binds hPTH in the sample;

contacting the sample with a second antibody or antibody fragment which selectively binds hPTH at an epitope contained within amino acids 24 to 37; wherein the second antibody or antibody fragment binds to hPTH bound by the first antibody or antibody fragment; and

detecting the binding of the first and second antibodies or antibody fragments, wherein the binding of the first and second antibodies or antibody fragments indicates the presence of active hPTH in the sample.

10. The method of claim 9, wherein the second antibody or antibody fragment selectively binds a peptide of hPTH selected from the group consisting of peptides having SEQ. ID. Nos. 18–36.

11. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 1.

12. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 2.

13. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 3.

14. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 4.

15. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 5.

16. The method of claim 9, wherein the first antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 6.

17. A composition comprising an antibody or antibody fragment and a suitable carrier, wherein the antibody or antibody fragment selectively binds a peptide of human parathyroid hormone (hPTH) selected from the group consisting of peptides having SEQ. ID. Nos. 1–6.

18. The composition of claim 17, wherein the composition further comprises a second antibody or antibody fragment, wherein the second antibody or antibody fragment selectively binds hPTH at an epitope contained within amino acids 24 to 37.

19. The composition of claim 17, wherein the second antibody or antibody fragment selectively binds a peptide of hPTH selected from the group consisting of peptides having SEQ. ID. Nos. 18–36.

20. The composition of claim 17, wherein the antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 1.

21. The composition of claim 17, wherein the antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 2.

22. The composition of claim 17, wherein the antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 3.

23. The composition of claim 17, wherein the antibody or antibody fragment selectively binds peptides of hPTH having SEQ. ID. No. 4.

24. The composition of claim 17, wherein the antibody or antibody fragment selectively binds peptides pf hPTH having SEQ. ID. No. 5.

25. The composition of claim 17, wherein the antibody or antibody fragment selectivley binds peptides of hPTH having SEQ. ID. No. 6.

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UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

NICHOLS INSTITUTE DIAGNOSTICS, INC., a California Corporation, Plaintiff, v. SCANTIBODIES CLINICAL LABORATORY, INC., a California Corporation; and SCANTIBODIES LABORATORY, INC., a California Corporation; and DOES 1 through 10, inclusive, Defendants.	No. 02 CV 0046 B (LAB) ANSWER AND COUNTERCLAIMS OF DEFENDANTS SCANTIBODIES CLINICAL LABORATORY, INC. AND SCANTIBODIES LABORATORY, INC.

Defendants Scantibodies Clinical Laboratory, Inc. and Scantibodies Laboratory, Inc. (collectively "Scantibodies") answer the Complaint for Patent Infringement of Nichols Institute Diagnostics, Inc. ("Nichols") as follows:

JURISDICTION AND VENUE

1. Scantibodies admits that the district court has subject matter jurisdiction over patent infringement actions, and that the Complaint attempts to set forth a claim for patent infringement.

2. Scantibodies admits that venue is proper in this jurisdiction for a properly pleaded patent infringement action against Scantibodies in that Scantibodies' principal place of business is located in this district and that Scantibodies conducts business in this district. Scantibodies denies each and every remaining allegation of paragraph 2 and further denies that it has committed any infringing acts within the district, or anywhere, as alleged in the Complaint.

PARTIES

3. On information and belief, Scantibodies admits the allegations of paragraph 3.

4. Scantibodies admits the allegations of paragraph 4.

5. Scantibodies admits the allegations of paragraph 5.

FIRST CLAIM

6. In response to paragraph 6, Scantibodies incorporates and realleges the above responses to paragraphs 1-5.

7. Scantibodies lacks the knowledge or information sufficient to form a belief as to the truth of the allegations of paragraph 7 and, therefore, denies each and every allegation contained therein. Scantibodies admits that Exhibit 1 purports to be a copy of U.S. Patent No. 6, 030, 790.

8. Scantibodies lacks the knowledge or information sufficient to form a belief as to the truth of the allegations of paragraph 8 and, therefore, denies each and every allegation contained therein.

9. Scantibodies denies each and every allegation contained in paragraph 9.

10. Scantibodies denies each and every allegation contained in paragraph 10.

11. Scantibodies denies each and every allegation contained in paragraph 11.

12. Scantibodies denies each and every allegation contained in paragraph 12.

13. Scantibodies denies each and every allegation contained in paragraph 13.

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AFFIRMATIVE DEFENSES

Based on the facts presently available to it, Scantibodies alleges the following affirmative defenses:

FIRST AFFIRMATIVE DEFENSE

(Failure to State a Claim)

14. The Complaint fails to state a claim upon which relief may be granted.

SECOND AFFIRMATIVE DEFENSE

(Laches/Estoppel/Waiver)

15. Nichols' Complaint, and any claims for damages alleged therein, are barred in whole or in part by the equitable doctrine of laches, estoppel and/or waiver.

THIRD AFFIRMATIVE DEFENSE

(Patent Invalidity: Anticipation/Obviousness)

16. Each of the claims of U.S. Patent No. 6, 030, 790 ("the '790 patent") is invalid on the grounds that the purported inventions attempted to be patented are invalid as anticipated under 35 U.S.C. § 102, or invalid as obvious under 35 U.S.C. § 103, or both.

FOURTH AFFIRMATIVE DEFENSE

(Estoppel)

17. By reason of the proceedings in the United States Patent Office, including the prosecution of the application which resulted in the '790 patent and related applications, and by reason of the elections, positions, concessions, representations, and statements therein taken or made by or on behalf of the applicant for such patents, Nichols is estopped from construing the claims of the patents at issue, even if this were otherwise possible, to cover and include any acts by Scantibodies.

FIFTH AFFIRMATIVE DEFENSE

(Patent Invalidity: Prior Invention)

18. Each of the claims of the patent in suit is invalid under 35 U.S.C. §§ 102(f) and 102(g).

SIXTH AFFIRMATIVE DEFENSE

(Patent Invalidity: 35 U.S.C. § 112)

19. Each of the claims of the patent in suit is invalid because the specification of the patent fails to comply with 35 U.S.C. § 112. The claims of the '790 patent are indefinite, and the patent is invalid for lack of enablement, failure to disclose the best mode, and inadequate written description.

SEVENTH AFFIRMATIVE DEFENSE

(Unenforceability By Reason of Inequitable Conduct)

20. The '790 patent is unenforceable because the inventors committed inequitable conduct before the United States Patent and Trademark Office with respect to the '790 patent application.

21. At the time of the filing of the application that issued as the '790 patent, the inventors were aware of information that was material to the patentability of the '790 patent claims. Material information which was known to at least some of the inventors, included at least the following: prior art references existed which disclosed elements or combinations of elements that were material to the examination of the application underlying the '790 patent; and what the inventors believed to be (1) the best mode for carrying out the claimed inventions at the time the application for the '790 patent was filed and (2) information relevant to enablement of the claimed inventions.

22. Pursuant to 37 C.F.R. § 1.56 ("Rule 56"), the inventors, their attorneys, and others substantially involved in the prosecution of a patent application are required to disclose to the Patent and Trademark Office (the "PTO") information available to them which is material to the patentability of the application.

23. The inventors, their attorneys, and others substantially involved in the prosecution of the '790 patent (1) failed to disclose the existence of prior art known to them which was material to the examination of the patentability of the '790 patent; (2) mischaracterized or misrepresented the content of material prior art references to the PTO;

(3) withheld from the PTO the best mode for carrying out the invention known to the inventors at the time the application for the '790 patent was filed; (4) withheld enabling information known to the applicants at the time the application was filed; and (5) failed to identify the correct inventors of the claimed inventions of the '790 patent to the PTO.

Failure to Disclose the Tampe, et al. Reference

24. The application for the '790 patent is entitled to a priority date in the United States of no earlier than September 22, 1995, the filing date of the PCT Application under 35 U.S.C. § 102(b). On information and belief, at the time they filed the application for the '790 patent, the inventors and their attorneys were aware of an article entitled "Characterization of Antibodies Against Human N-Terminal Parathyroid Hormone by Epitope Mapping" published in *Journal of Immunoassay* 13(1) 1-13 (1992), by Jens Tampe, *et al.* The article, which was published in 1992, is § 102(b) prior art to the '790 patent.

25. The inventors and their attorneys were aware of the Tampe article and its materiality, as they included it in the background discussion of the '790 patent specification. The article was clearly material to the patentability of the claimed inventions, as the PTO Examiner cited it as the bases for rejecting all pending claims in an August 13, 1998 office action.

26. Neither the inventors nor their agents filed an Information Disclosure Statement ("IDS"), or a PTO Form 1449, with the PTO at any time during the prosecution of the application that issued as the '790 patent. As a result, the inventors and their attorneys never disclosed the content of the Tampe article to the PTO in connection with the examination of the application for the '790 patent.

27. That failure constitutes a breach of the inventors' duty, pursuant to Rule 56, to disclose to the PTO information available to them which is material to the patentability of the application.

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Misrepresentations to the PTO About the Tampe Reference

28. During the prosecution of the '790 patent, the PTO Examiner issued an office action on August 13, 1998, in which she rejected the pending claims of the application in light of the Tampe *et al.* reference.

29. Applicants, through their attorneys, responded to the office action on December 14, 1998. On pages 8-9 of their response, Applicants wrote: "Applicant respectfully submit that Tampe *et al* fail to teach antibodies and antibody fragments which selectively bind to active hPTH or antibodies and antibody fragments that are capable of binding to the N-terminus of hPTH." On that basis, Applicants misrepresented that Tampe *et al* "fail to teach each element in the currently pending claims."

30. Applicants further misrepresented the teachings in Tampe *et al* in the background discussion of the '790 patent. In Column 2, lines 21-24, of the patent, Applicants, through their attorneys, included the following statement about the Tampe reference in the original application to the PTO: "However, these antibodies are not capable of discriminating between biologically active and biologically inactive PTH (1-84) or fragments thereof lacking the first two amino acids serine and valine."

31. Both the office action response and the discussion in the specification constitute misrepresentations of the Tampe reference. The Tampe *et al* reference clearly teaches antibodies and antibody fragments capable of selectively binding to the N-terminus of hPTH.

32. On information and belief, the Applicants and their attorneys were aware of this teaching in the Tampe reference.

33. On information and belief, the misrepresentations contained in the office action response dated December 14, 1998 were made with an intent to deceive the PTO. The misrepresentations were highly material, since they opposed an argument of unpatentability from the PTO.

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Concealing Material Information Related to Enablement and the Best Mode For Carrying Out the Claimed Invention

34. In their own 1998 article (prior to the issuance of the '790 patent) entitled "Production of Sequence Specific Polyclonal Antibodies to Human Parathyroid Hormone 1-37 by Immunization With Multiple Antigenic Peptides," Applicants discussed the generation of site-specific antibodies to the N-terminus of hPTH peptide, e.g., hPTH 1-10, by incorporating certain amino acid residues and using "multiple antigenic peptide systems (MAP) for immunization." The article discussed the failure of other techniques, such as those disclosed and claimed in the '790 patent, to generate site-specific antibodies to the N-terminus of hPTH.

35. Neither the Applicants nor their attorneys ever disclosed Applicants' 1998 article to the PTO Examiner.

36. In their 1998 article, Applicants referred to their own prior work using the MAP technique, as disclosed in their own 1994 article. The 1994 article, written by Applicants, pre-dates the September 22, 1995 filing date for the application underlying the '790 patent.

37. Neither the Applicants nor their attorneys ever disclosed use of the MAP technique for generating site-specific antibodies to the N-terminus of hPTH, the purported invention claimed by the '790 patent to the PTO.

38. Applicants knew of the MAP technique to generate site-specific antibodies to the N-terminus of hPTH at least as early as September 22, 1995, the filing date of the PCT Application (WO 96/10041). Applicants disclosed the MAP technique in the specification of the PCT Application, as well as in the European Patent Application (EP 0 783 522 B1) that was filed simultaneously with the application in the United States for the '790 patent. Both the '790 patent and the European patent application claimed priority from the PCT application. At the time they filed their application for the '790 patent in the United States, Applicants knew of the MAP technique and knew it was the best mode for carrying out their

claimed invention. However, Applicants failed to disclose the MAP technique to the PTO in connection with the application for the '790 patent.

Concealing Identity of the True Inventors from the PTO

39. On information and belief, applicants for the '790 patent concealed from the PTO the true identity of all the correct inventors of the invention claimed in the patent.

40. Scantibodies is informed and believes, and on that basis alleges, that Wolf-Georg Forssmann and others made inventive contributions to the '790 patent.

41. Forssmann was listed as an inventor on the PCT Application (WO 96/10041) and the European Patent Application (EP 0 783 522 B1) that relate to the subject matter disclosed and claimed in the '790 patent. Forssmann also was listed as an inventor on the 1994 German Patent Application (P 44 34 551). That application is the purported basis of the foreign application priority date for the '790 patent. The PCT Application and the European Patent Application also both claimed priority from German Application No. P 44 34 551.

42. On information and belief, applicants, their attorneys, and others substantially involved in the prosecution of the '790 patent, were aware of the identity of the correct inventors.

43. Applicants, their attorneys, and others substantially involved in the prosecution of the '790 patent never disclosed to the PTO the identity of the correct inventors.

44. On information and belief, the failure to identify the correct inventors of the '790 patent was done with an intent to deceive the PTO. The information related to the identity of the correct inventors was highly material to the PTO.

EIGHTH AFFIRMATIVE DEFENSE

(Inventorship)

45. Each of the claims of the '790 patent is invalid under 35 U.S.C. §§ 102(f), 111 and 116 for failure to name all of the correct inventors.

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COUNTERCLAIMS

By way of counterclaims against Nichols, Scantibodies alleges as follows:

JURISDICTION AND VENUE

46. This Court has jurisdiction over this matter pursuant to 28 U.S.C. §§ 1331 and 1338(a), because this is an action arising under the federal patent laws as to which the district courts have original jurisdiction. Venue is proper in this district pursuant to 28 U.S.C. § 1391(b) because the defendant resides in this judicial district.

47. This Court has original jurisdiction of the Second Counterclaim under 28 U.S.C. § 1338(b) because the Second Counterclaim is an unfair competition claim joined with a substantial and related claim under the patent laws of the United States. This Court has supplemental jurisdiction of the Second Counterclaim under 28 U.S.C. § 1367 because it is so related to Nichols' federal claim that it forms a part of the same case or controversy under Article III of the United States Constitution.

48. The defendant-counterplaintiffs, Scantibodies Clinical Laboratory, Inc. and Scantibodies Laboratory, Inc. (collectively "SCL") are corporations organized and existing under California law, having principal place of business in Santee, California.

49. On information and belief, the plaintiff-counterdefendant Nichols Institute Diagnostics, Inc. ("Nichols") is a corporation organized and existing under California law and has its principal place of business in San Juan Capistrano, California.

STATEMENT OF THE CASE

50. The counterclaims asserted herein are predicated on Nichols' anticompetitive, exclusionary and unfair conduct. Nichols is engaging in unfair competition in violation of California Business and Professions Code Section 17200 *et seq.*

PTH TEST KIT INDUSTRY AND MARKET

51. Parathyroid hormone (PTH) is an 84 amino acid peptide. It is a major factor in the calcium metabolism of the human body. The measurement of PTH in plasma has been central to laboratory investigation of patients with calcium metabolism disorders.

52. Generation of site-specific antibodies to the N-terminal bioactive fragment of PTH is key to the development of assays for measurement of PTH.

53. Due to the importance of detecting accurate levels of PTH in patients, such as kidney dialysis patients, a market has developed for these PTH assays and PTH test kits.

54. A domestic market for these PTH test kits has developed in the United States. The market continues to grow every year. Worldwide sales of PTH test kits in 2000 totaled approximately $30 million. In 2001, that amount increased to approximately $33 million.

55. Nichols is the largest producer of PTH test kits in the United States for the kidney dialysis patient testing market. In 2001, Nichols had approximately 75 % of the kidney dialysis patient testing market share in the United States. Behind Nichols, the next largest market shares for the PTH test kit market belonged to the following vendors: Diagnostic Products Corporation (DPC), DiaSorin, and Diagnostic Systems Laboratory (DSL). Scantibodies has been and continues to be a participant in the PTH test kit and lab services market in the United States.

56. Nichols has monopoly and/or market power in the PTH test kit market in the United States for kidney dialysis patient testing. Scantibodies is informed and believes, and on that basis alleges, that Nichols controls approximately 75 % or more of the PTH test kit market in the United States for kidney dialysis patient testing.

FDA REGULATIONS GOVERNING PTH TESTING

57. The extensive regulation by the FDA of medical devices includes the regulation of "in vitro diagnostic products." *See* 21 CFR § 801.119. Test kits for PTH levels in serum and plasma (processed human blood) fall within the definition of "in vitro diagnostic products" subject to FDA regulation as set forth in 21 CFR § 809.3. The applicable federal regulations classify test kits for PTH levels in humans as class two clinical chemistry and clinical toxicology devices pursuant to 21 CFR § 862.1545.

58. As class two in vitro diagnostic products, all test kits for PTH are subject to the FDA requirements for premarket notification in 21 CFR § 807.81, *et seq.*, and labeling in 21 CFR § 809.10. The premarket notification requirement mandates that all PTH test kit

manufactures provide premarket notification to FDA before introducing PTH test kits into interstate commerce. The labeling requirement mandates that every PTH test contain a label (or manufacturer's instructions or package insert) that includes, among other things, "[r]ecommended storage, handling or shipping instructions for the protection and maintenance of stability of the specimen" to be tested. 21 CFR § 809.10(b)(7)(iv). The manufacturer's instructions or package insert is submitted to the FDA as part of the premarket notification process.

59. Laboratories that use the NICHOLS PTH test kits are required to ensure that certain quality controls are maintained in the use of PTH test kits subject to FDA regulation pursuant to 42 CFR § 493 *et seq*. These quality control requirements mandate that, prior to reporting patient test results, the laboratories must either: (1) follow the PTH test kit manufacturer's instructions or package insert under 42 CFR § 493.1202(c); or (2) for each method that deviates from the manufacturer's instruction or package insert, comply with 42 CFR § § 493.1201 through 493.1221, including verifying and establishing the accuracy, precision, analytical sensitivity, analytical specificity, reportable range of patient test results, reference ranges and any other performance characteristics required for test performance under 42 CFR § 493.1213; establishing calibration and calibration verification procedures for patient testing under 42 CFR § 493.1217, establishing control procedures under 42 CFR § 493.1218; and maintaining documentation of the verification or establishment of all applicable test performance specifications and quality control activities under CFR § 493.1213(c) and 42 CFR § 493.1221. On information and belief, no laboratory that uses the NICHOLS PTH test kit has complied with the requirements of 42 CFR § § 493.1201 through 493.1221 as required to entitle it to modify the NICHOLS' manufacturer's instructions when using the NICHOLS PTH test kit.

60. A manufacturer of a PTH test kit, such as NICHOLS, is required when it knows, or has knowledge of facts that would give it notice, that its PTH test kit is to be used for conditions, purposes, or uses other than the ones for which the test kit was offered when

the manufacturer sold it, to re-label the test kit for the new intended use pursuant to 21 CFR § 801.4.

THE NICHOLS MANUFACTURER'S INSTRUCTIONS OR PACKAGE INSERT

61. NICHOLS has submitted to the FDA, on at least three occasions (the most recent in December 2001), a premarket notification for a PTH test kit pursuant to Section 510(k) of the Federal Food, Drug and Cosmetic Act. For each of these premarket notifications, NICHOLS submitted to the FDA its manufacturer's instructions or package insert for its PTH test kit that is distributed to NICHOLS customers to comply with the labeling requirements of 21 CFR § 809.10.

62. The NICHOLS' manufacturer's instructions or package insert requires that whole blood samples drawn from dialysis patients for PTH testing: (1) be processed to remove cells by centrifugation (or "spinning") "as soon as possible" into serum or plasma; (2) be stored after collection and centrifugation at room temperature for a maximum of 2 hours as serum or 48 hours as plasma; (3) be stored after collection and centrifugation at 4 degrees Celsius for a maximum of 6 or 8 hours as serum; or (4) be frozen either as serum or plasma immediately following collection and centrifugation. There is no allowance for storing samples for PTH testing as unprocessed whole blood.

63. Processing of the whole blood samples taken from dialysis patients for PTH testing into serum or plasma requires that the whole blood be "spun" in a centrifuge to separate the plasma from the red blood cells. The processing or "spinning" of the whole blood samples must occur before the sample is frozen. The NICHOLS' manufacturer's instructions or package insert for its PTH test kits requires that the whole blood samples be immediately processed ("spun") and stored as plasma or serum according to the specific temperature and time requirements outlined in paragraph 62 above.

NICHOLS' INDUCEMENT OF THE VIOLATION OF FDA REGULATIONS BY ITS LABORATORY CUSTOMERS

64. Despite the instruction in its manufacturer's instructions or package insert that whole blood be immediately processed ("spun") into plasma or serum, and frozen within 6 or

8 hours after collection (for serum) and stored at room temperature for a maximum of 48 hours after collection (for plasma), NICHOLS, in combination with a laboratory customer (LifeChem Laboratory Services), published an abstract in the scientific journal *Clinical Chemistry* in 1995 to induce its laboratory customers to wait up to 24 hours before processing whole blood samples for PTH testing. The abstract further advised that following processing (centrifugation), plasma or serum samples could be stored at room temperature for 24, 48 or even 72 hours before testing.

65. In the *Clinical Chemistry* abstract, NICHOLS claimed to have conducted a study in which blood samples (not centrifuged and separated) were held at room temperature for "24 hours to simulate typical shipping conditions" from dialysis centers to laboratories. These whole blood samples were "spun" or processed into plasma after 24 hours at room temperature, and allowed to remain at room temperature an additional 24, 48 and 72 hours before being tested for PTH. The results from testing these samples were then compared against the results from testing "frozen serum specimens" taken from the same patients.

66. The result from this research advocated by Defendants NICHOLS was that allowing whole blood samples to remain at room temperature for 24 hours before processing into plasma or serum — that is, allowing unprocessed whole blood samples to remain at room temperature "for shipment" from dialysis centers to its customers' laboratories — produced results with a correlation of "less than one," but found that this lack of correlation was not statistically significant, permitting the purported conclusion that whole blood could be held at room temperature for 24 hours without centrifugation and separation, to allow for shipping from dialysis centers to its laboratory customers, before being processed into plasma or serum.

67. One of the authors of this *Clinical Chemistry* abstract, who is currently employed by NICHOLS, boasted in March 2002 that the alleged study in this abstract saved Defendant NICHOLS' laboratory customers large amounts of money by eliminating the cost and expense of processing or "spinning" the whole blood samples at their dialysis centers,

and shipping frozen plasma or serum samples packed in dry ice from the dialysis centers to the laboratory for PTH testing.

NICHOLS' LABORATORY CUSTOMERS' VIOLATION OF THE NICHOLS' MANUFACTURER'S INSTRUCTIONS

68. As a direct result of NICHOLS' inducement, several of its major laboratory customers (representing PTH testing for over 50% of the approximately 330,000 dialysis patients in the United States) who purchase and use the NICHOLS' PTH test kits, provide written instructions to their own dialysis center employees that violate the NICHOLS PTH test kit manufacturer's instructions or package insert regarding the processing and storage of whole blood for PTH testing. Specifically, these instructions direct employees at the dialysis centers, that are either owned or controlled by the laboratories, not to process (spin) whole blood samples immediately as required by the NICHOLS' manufacturer's instructions and package insert NICHOLS submitted to the FDA.

69. The written instructions NICHOLS' laboratory customers provide to dialysis centers, while directly contrary to the NICHOLS' PTH test kit manufacturer's instructions and package insert, are consistent with the inducement provided by NICHOLS in the 1995 *Clinical Chemistry* abstract that whole blood samples not be processed (centrifuged and separated) for up to 24 hours or assayed for up to 72 hours after collection. These instructions allow NICHOLS' laboratory customers to avoid the expense and cost of processing the whole blood at the dialysis centers and shipping the processed serum or plasma in dry ice to their laboratories. These instructions also unfairly impede laboratories, such as Scantibodies, that require compliance with their manufacturer's instructions -- dictating that whole blood samples be processed into serum or plasma before shipment to a laboratory for PTH testing -- from competing in the PTH testing and lab services market.

70. Despite NICHOLS' laboratory customers' failure to comply with its manufacturer's instructions or package insert for its PTH test kits, and NICHOLS' knowledge of and inducement of this non-compliance by its laboratory customers, NICHOLS has not re-labeled its PTH test kit, and continued to submit manufacturer's instructions or package

inserts for its PTH test kits to the FDA in premarket notifications that violate the applicable labeling and intended use regulations.

FIRST COUNTERCLAIM

(Declaratory Judgment of Invalidity and Non-Infringement)

71. Scantibodies incorporates and realleges paragraphs 1-70 of its Answer, Affirmative Defenses and Counterclaims as if fully set forth herein.

72. This claim arises under the Federal Declaratory Judgment Act and the Patent Laws of the United States, and more particularly, under 28 U.S.C. §§ 2201 and 2202, and 35 U.S.C. § 101 et seq.

73. There exists a justiciable controversy between Scantibodies and Nichols concerning whether the '790 patent is valid and enforceable, and if so, whether Scantibodies is liable to Nichols for infringement of any of the claims of the patent at issue.

74. Scantibodies seeks a declaration that the '790 patent is invalid and/or unenforceable.

75. In addition, or as further relief, Scantibodies seeks a declaration that Nichols is estopped from enforcing the '790 patent based on its own wrongful conduct and/or is barred from collecting damages under the patent due to laches, waiver, acquiescence or and/or misuse.

76. A judicial declaration is necessary and appropriate at this time in order that Scantibodies may ascertain its rights and duties with respect to the patent in suit.

SECOND COUNTERCLAIM

(Violation of California Business & Professions Code Section 17200)

77. Scantibodies incorporates and realleges paragraphs 1-76 of its Answer, Affirmative Defenses and Counterclaims as if fully set forth herein.

78. Scantibodies brings this claim in both its individual capacity and on behalf of the general public pursuant to California Business & Professions Code Section 17204.

79. Beginning in at least 1995 and continuing to the present, NICHOLS has committed acts of unfair competition, as that term is defined in California Business &

Professions Code Section 17200, by engaging in the following unlawful, unfair and fraudulent business acts: (1) failing to comply with the labeling requirements of 21 CFR § 809.10 for the "storage, handling or shipping instructions for the protection and maintenance of stability" of blood samples collected from dialysis patients for PTH testing at its customers' laboratories; (2) failing to re-label its PTH test kits when it knew, or had knowledge of facts that gave it notice, that its PTH test kit was being used for conditions, purposes, or uses other than the ones for which the test kit was offered as stated in NICHOLS' manufacturer's instructions or package insert, in violation of 21 CFR § 801.4; and (3) inducing its laboratory customers to violate 42 CFR § 493 *et seq.* by inducing them to not follow the NICHOLS' manufacturer's instructions or package insert when storing and shipping samples collected from dialysis patients for PTH testing. These violations of the federal regulations controlling the labeling of NICHOLS' PTH test kits constitute unlawful, unfair and fraudulent business practices within the meaning of California Business & Professions Code Section 17200.

80. The harm to Scantibodies and to members of the general public outweighs the utility of Nichols' practice of failing to properly relabel its package insert or manufacturer's instructions to reflect the actual practice of its laboratory customers in processing and storing samples taken from dialysis patients for PTH testing, and therefore constitutes an unfair practice within the meaning of Business & Professions Code Section 17200.

81. Nichols' unlawful, unfair and fraudulent business practices, as described above, continue in that Nichols continues to sell its PTH test kits with the knowledge that its laboratory customers are failing to comply with its package insert or manufacturer's instructions for processing and storing patient samples. Scantibodies and other members of the public do have not an adequate remedy at law.

82. As a direct result of the above-described unlawful, unfair and fraudulent acts, NICHOLS has continued to dominate the sale of PTH test kits in California, and elsewhere, and also hindered Scantibodies' competition in the market for PTH testing and lab services. This has

resulted in NICHOLS obtaining and holding ill-gotten gains through the sale of PTH test kits to its laboratory customers.

WHEREFORE, Scantibodies prays for judgment that:

On Nichols' Complaint:

(a) Nichols take nothing by way of its Complaint;

(b) Scantibodies does not infringe the '790 patent;

(c) the patents at issue are invalid and/or unenforceable against Scantibodies;

(d) Nichols is estopped from asserting that the patents at issue are infringed by Scantibodies;

(e) Nichols is barred from collecting damages under the '790 patent based on laches, waiver, acquiescence and/or misuse;

(f) Scantibodies be awarded its costs and attorney's fees; and

(g) Scantibodies be awarded such other and further relief as the Court may deem just and reasonable.

On Scantibodies' Counterclaims:

WHEREFORE, the defendant-counterplaintiff Scantibodies respectfully requests this court to find and rule:

(a) That, with respect to the First Counterclaim, the Court enter a declaratory judgment that the Scantibodies products do not infringe any of the claims of the '790 patent, and that

(i) the '790 patent is invalid;

(ii) the '790 patent is unenforceable against Scantibodies;

(iii) Nichols is estopped from asserting that the patent at issue is infringed by Scantibodies;

(iv) Nichols is barred from collecting damages under the '790 patent based on laches, waiver, acquiescence and/or misuse;

(v) Scantibodies be awarded its costs and attorney's fees; and

(vi) such other relief that this Court deems just and reasonable.

(b) That, with respect to the Second Counterclaim, the following relief be granted:

(i) Nichols be enjoined from engaging in the unlawful, unfair and fraudulent business practices stated in Paragraph 79, *supra*, pursuant to California Business & Professions Code Sections 17203 and the equitable powers of this Court;

(ii) Nichols be ordered to pay restitution of monies obtained from its unlawful, unfair and fraudulent business practices stated in Paragraph 79, *supra*, pursuant to California Business & Professions Code Sections 17203 and the equitable powers of this Court;

(iii) For attorneys fees pursuant to California Code of Civil Procedure Section 1021.5; and

(iv) For costs of suit, and for such further relief as the Court may order.

Scantibodies requests a jury trial as to its counterclaims.

Dated: May 9, 2002

DAVID C. DOYLE
ERIC M. ACKER
M. ANDREW WOODMANSEE
SHANNON M. DAILEY
MORRISON & FOERSTER LLP

By: David C. Doyle
David C. Doyle

Attorneys for Defendants
SCANTIBODIES CLINICAL LABORATORY, INC. AND SCANTIBODIES LABORATORY, INC.

DAVID C. DOYLE (BAR NO. 70690)
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UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

NICHOLS INSTITUTE DIAGNOSTICS, INC., a California Corporation, Plaintiff, v. SCANTIBODIES CLINICAL LABORATORY, INC., a California Corporation; and SCANTIBODIES LABORATORY, INC., a California Corporation; and DOES 1 through 10, inclusive, Defendants.	No. 02 CV 0046 B (LAB) SUBSTITUTION OF COUNSEL BY DEFENDANTS SCANTIBODIES CLINICAL LABORATORY, INC. AND SCANTIBODIES LABORATORY, INC.

Pursuant to Civ. L.R. 83.3, defendants Scantibodies Clinical Laboratory, Inc. and Scantibodies Laboratory, Inc. (collectively "Scantibodies"), hereby substitute David C. Doyle (Bar No. 70690), Morrison & Foerster LLP, 3811 Valley Centre Drive, Suite 500, San Diego, California 92130 as attorney of record in place of Luce, Forward, Hamilton & Scripps LLP.

Dated: May 9, 2002

By:_____
Thomas Cantor
President & CEO, Scantibodies Clinical Laboratory, Inc. and Scantibodies Laboratory, Inc.

Dated: May 9, 2002

By: _____
Thomas Cantor
President & CEO, Scantibodies Clinical Laboratory, Inc. and Scantibodies Laboratory, Inc.

I hereby consent to the above substitution of attorney.

Dated: May 9, 2002

By: _____
Edward Patrick Swan
LUCE, FORWARD, HAMILTON & SCRIPPS

I am duly admitted to practice in this District and consent to substitution as attorney of record.

Dated: May 9, 2002

MORRISON & FOERSTER LLP

By: _____
David C. Doyle

Substitution of attorney is hereby approved.

Dated: _____, 2002

_____
Honorable Rudi M. Brewster
United States District Judge

I hereby consent to the above substitution of attorney.

Dated: May 9, 2002

By: _____
Edward Patrick Swan, Jr.
LUCE, FORWARD, HAMILTON & SCRIPPS

I am duly admitted to practice in this District and consent to substitution as attorney of record.

Dated: May 9, 2002

MORRISON & FOERSTER LLP

By: _____
David C. Doyle

Substitution of attorney is hereby approved.

Dated: _____, 2002

_____
Honorable Rudi M. Brewster
United States District Judge

I hereby consent to the above substitution of attorney.

Dated: May 9, 2002

By: _____
Edward Patrick Swan
LUCE, FORWARD, HAMILTON & SCRIPPS

I am duly admitted to practice in this District and consent to substitution as attorney of record.

Dated: May 9, 2002

MORRISON & FOERSTER LLP

By: David C. Doyle
David C. Doyle

Substitution of attorney is hereby approved.

Dated: _____, 2002

_____
Honorable Rudi M. Brewster
United States District Judge

Dated: May 9, 2002

DAVID C. DOYLE
ERIC M. ACKER
M. ANDREW WOODMANSEE
SHANNON M. DAILEY
MORRISON & FOERSTER LLP

By: David C. Doyle
David C. Doyle

Attorneys for Defendants
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Attorneys for Defendants
SCANTIBODIES CLINICAL LABORATORY, INC. AND SCANTIBODIES LABORATORY, INC.

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

NICHOLS INSTITUTE DIAGNOSTICS, INC., a California Corporation, Plaintiff, v. SCANTIBODIES CLINICAL LABORATORY, INC., a California Corporation; and SCANTIBODIES LABORATORY, INC., a California Corporation; and DOES 1 through 10, inclusive, Defendants.	No. 02 CV 0046 B (LAB) PERSONAL PROOF OF SERVICE

I, the undersigned, declare as follows:

1. At the time of service I was at least 18 years of age and not a party to this case, and I served copies of the following documents:

ANSWER AND COUNTERCLAIMS OF DEFENDANTS SCANTIBODIES CLINICAL LABORATORY, INC. AND SCANTIBODIES LABORATORY, INC.

SUBSTITUTION OF COUNSEL BY DEFENDANTS SCANTIBODIES CLINICAL LABORATORY, INC. AND SCANTIBODIES LABORATORY, INC.

a. Party served: **Nichols Institute Diagnostics, Inc.**
b. Person served: **Douglas E. Olson, Attorney for Plaintiff**

c. Place of service (Business)

F.T. Alexandra Mahaney
Moana L. McMullan
BROBECK, PHLEGER & HARRISON, LLP
12390 El Camino Real
San Diego, CA 92130-2081
Telephone: (858) 720-2500
Facsimile: (858) 720-3700

3. I served the party named in item 2

a. ☒ by personally delivering the copies on May 9, 2002 at 2:24 p.
b. ☐ by leaving the copies at the attorney's office, in an envelope or package clearly labeled to identify the attorney being served,
☒ with a receptionist or, with a person having charge thereof.
☐ in a conspicuous place in the office between the hours of nine in the morning and five in the afternoon.

c. ☐ by leaving the copies at the individual's residence with some person of not less than 18 years of age. (If service was to a party and not an attorney, delivery was made between the hours of 8:00 a.m. and 6:00 p.m.).

4. Person serving (name, business address, and telephone no.:

ASAP Legal Courier, Inc.
4250 Pacific Highway, Suite 120
San Diego, CA 92110
Tel: (619) 221-0700
Fax: (619) 221-0760

a. Fee for service: $
b. ☐ Not a registered California process server.
c. ☐ Exempt from registration under B&P 22350(b).
d. ☑ Registered California process server.
(1) ☑ Employee or independent contractor.
(2) Registration No.:
(3) County:

☒ I declare under penalty of perjury under the laws of the State of California that the foregoing is true and correct.

Date: 05/09/02 (Signature)

****
*** TX REPORT ***
****

TRANSMISSION OK

TX/RX NO	2816
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RESULT	OK

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Transmission problems: (858) 720-2657

Brobeck
ATTORNEYS AT LAW

May 10, 2002

Brobeck, Phleger & Harrison LLP
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www.brobeck.com

PAGES TO FOLLOW COVER	25	USER ID	6157	CMID NUMBER	0033091.0002

TO

RECIPIENT	COMPANY	TELEPHONE	FAX
Wayne B. Brown, Esq.	Quest Diagnostics, Inc.	(949) 728-4747	(949) 728-4930
Melissa Margulies	Quest Diagnostics, Inc.	(610) 454-4148	(610) 983-2138

FROM

SENDER	EMAIL	TELEPHONE	FAX
Douglas E. Olson	dolson@brobeck.com	(858) 720-2662	(858) 720-3700

MESSAGE

Please see attached:

FACSIMILE TRANSMISSION

Transmission problems: (858) 720-2657

Brobeck

ATTORNEYS AT LAW

May 10, 2002

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Melissa Margulies	Quest Diagnostics, Inc.	(610) 454-4148	(610) 983-2138

FROM

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Douglas E. Olson	dolson@brobeck.com	(858) 720-2662	(858) 720-3700

MESSAGE

Please see attached:

****
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****

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TX/RX NO	1714
CONNECTION TEL	033091#0002#19497284930#
CONNECTION ID	
ST. TIME	05/10 15:41
USAGE T	09'44
PGS. SENT	26
RESULT	OK

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Transmission problems: (858) 720-2657

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May 10, 2002

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FROM

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MESSAGE

Please see attached:

15

DAVID C. DOYLE (BAR NO. 70690)
ERIC M. ACKER (BAR NO. 135805)
M. ANDREW WOODMANSEE (BAR NO. 201780)
SHANNON M. DAILEY (BAR NO. 185634)
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Attorneys for Defendants
SCANTIBODIES CLINICAL LABORATORY, INC. and SCANTIBODIES LABORATORY, INC.

UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

NICHOLS INSTITUTE DIAGNOSTICS, INC., a California corporation,

Plaintiff,

v.

SCANTIBODIES CLINICAL LABORATORY, INC., a California corporation; and SCANTIBODIES LABORATORY, INC., a California corporation,

Defendants.

SCANTIBODIES CLINICAL LABORATORY, INC., a California corporation; and SCANTIBODIES LABORATORY, INC., a California corporation,

Counter-Claimants

v.

NICHOLS INSTITUTE DIAGNOSTICS, INC., a California corporation,

Counter-Defendants.

No. 02 CV 0046 B (LAB)

NOTICE OF MOTION AND MOTION FOR SUMMARY JUDGMENT PURSUANT TO 35 U.S.C. § 102(f) FOR NONJOINDER OF CO-INVENTOR

[FED. R. CIV. P. 56]

Date: July 15, 2002
Time: 10:30 a.m.
Courtroom 2

Hon. Rudi M. Brewster

TO PLAINTIFF NICHOLS INSTITUTE DIAGNOSTICS, INC., AND ITS ATTORNEY OF RECORD:

PLEASE TAKE NOTICE THAT on July 15, 2002 at 10:30 a.m., or as soon thereafter as the matter may be heard, in Courtroom 2 of the United States District Court for the Southern District of California located at 940 Front Street, San Diego, California, 92101, before the Honorable Rudi M. Brewster, Defendants Scantibodies Clinical Laboratory, Inc. and Scantibodies Laboratory, Inc. ("Scantibodies") will move, and hereby do move, for summary judgment against Plaintiffs.

The grounds for this motion are:

(1) The applicants for U.S.Patent No. 6,030,790 failed to identify and join all the inventors in their application to the United States Patent and Trademark Office;

(2) The '790 patent lists only three co-inventors on its face: Knut Adermann ("Adermann"), Dieter Hock ("Hock"), and Markus Mägerlein ("Mägerlein"). The '790 is the "national stage" of an earlier international patent application filed under the Patent Cooperation Treaty ("PCT"). As was appropriate under the PCT and the patent laws of the United States, '790 claimed the filing date of the earlier PCT Application. The PCT Application *is* the application for what ultimately issued as the '790 patent;

(3) Although the '790 patent originated in the PTO as the PCT Application -- and necessarily relied on the same disclosure -- the '790 patent does not name the same inventors. The PCT Application identified Adermann, Hock, Mägerlein *and Wolf-Georg Forssmann* as inventors. The '790, however, does not include Forssmann; and

(4) The omission of Forssmann from the national stage application in the United States renders the '790 patent invalid under 35 U.S.C. § 102(f) for nonjoinder of a co-inventor.

This motion is made pursuant to Rule 56 of the Federal Rules of Civil Procedure and is based upon this notice of motion and motion, the accompanying memorandum of points and authorities, the declaration of M. Andrew Woodmansee, the other pleadings and papers

on file in this action, and such other evidence and argument as may subsequently be presented to the Court.

Dated: May 15, 2002

DAVID C. DOYLE
ERIC M. ACKER
M. ANDREW WOODMANSEE
SHANNON M. DAILEY
MORRISON & FOERSTER LLP

By: David C. Doyle
David C. Doyle

Attorneys for Defendants
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SCANTIBODIES LABORATORY, INC.

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UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

NICHOLS INSTITUTE DIAGNOSTICS, INC., a California corporation, Plaintiff, v. SCANTIBODIES CLINICAL LABORATORY, INC., a California corporation; and SCANTIBODIES LABORATORY, INC., a California corporation, Defendants.	No. 02 CV 0046 B (LAB) **PROOF OF SERVICE**
SCANTIBODIES CLINICAL LABORATORY, INC., a California corporation; and SCANTIBODIES LABORATORY, INC., a California corporation, Counter-Claimants v. NICHOLS INSTITUTE DIAGNOSTICS, INC., a California corporation, Counter-Defendants.	

I, the undersigned, declare that I am employed with the law firm of Morrison & Foerster LLP, whose business address is 3811 Valley Centre Drive, Suite 500, San Diego, California 92130-2332. I am over the age of eighteen years and not a party to the within action. On May 16, 2002, I served the documents named below on the parties in this action as follows:

NOTICE OF MOTION AND MOTION FOR SUMMARY JUDGMENT PURSUANT TO 35 U.S.C. § 102(f) FOR NONJOINDER OF CO-INVENTOR

SCANTIBODIES CLINICAL LABORATORY, INC. AND SCANTIBODIES LABORATORY, INC.'S MEMORANDUM OF POINTS AND AUTHORITIES IN SUPPORT OF MOTION FOR SUMMARY JUDGMENT PURSUANT TO 35 U.S.C. § 102(f) FOR NONJOINDER OF CO-INVENTOR

DECLARATION OF M. ANDREW WOODMANSEE IN SUPPORT OF SCANTIBODIES CLINICAL LABORATORY, INC. AND SCANTIBODIES LABORATORY, INC.'S MOTION FOR SUMMARY JUDGMENT PURSUANT TO 35 U.S.C. § 102(f) FOR NONJOINDER OF CO-INVENTOR

[PROPOSED] ORDER GRANTING MOTION FOR SUMMARY JUDGMENT PURSUANT TO 35 U.S.C. § 102(f) FOR NONJOINDER OF CO-INVENTOR

Douglas E. Olson
F.T. Alexandra Mahaney
Moana L. McMullan
BROBECK, PHLEGER & HARRISON
12750 High Bluff Drive, Suite 300
San Diego, CA 92130-2081
Telephone: (858) 720-2500
Facsimile: (858) 720-3700

Attorneys for Plaintiff
NICHOLS INSTITUTE DIAGNOSTICS, INC.

☐ (BY MAIL) I caused each such envelope, with postage thereon fully prepaid, to be placed in the United States mail at San Diego, California. I am readily familiar with the practice of Morrison & Foerster LLP for collection and processing of correspondence for mailing, said practice being that in the ordinary course of business, mail is deposited in the United States Postal Service the same day as it is placed for collection.

☒ (BY PERSONAL SERVICE) I delivered to an authorized courier or driver authorized by ASAP Courier, Inc., 4250 Pacific Highway, Suite 120, San Diego, California 92110, to receive documents to be delivered on the same date. A proof of service signed by the authorized courier will be filed with the court upon request.

☐ (BY FEDERAL EXPRESS) I am readily familiar with the practice of Morrison & Foerster LLP for collection and processing of correspondence for overnight delivery and know that the document(s) described herein will be deposited in a box or other facility regularly maintained by Federal Express for overnight delivery.

☐ (BY FACSIMILE) The above-referenced document was transmitted by facsimile transmission and the transmission was reported as complete and without error. The facsimile machine I used complied with California Rules of Court, Rule 2003(3) and no error was reported

by the machine. Pursuant to California Rules of Court, Rule 2006(d), I caused the machine to print a transmission record of the transmission, a copy of which is attached to this declaration.

I declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct, and that this declaration is executed on May 16, 2002 at San Diego, California.

Kirsten Blue
(Printed)

(Signature)

16

DAVID C. DOYLE (BAR NO. 70690)
ERIC M. ACKER (BAR NO. 135805)
M. ANDREW WOODMANSEE (BAR NO. 201780)
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Attorneys for Defendants
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UNITED STATES DISTRICT COURT

SOUTHERN DISTRICT OF CALIFORNIA

NICHOLS INSTITUTE DIAGNOSTICS, INC., a California corporation,

Plaintiff,

v.

SCANTIBODIES CLINICAL LABORATORY, INC., a California corporation; and SCANTIBODIES LABORATORY, INC., a California corporation,

Defendants.

SCANTIBODIES CLINICAL LABORATORY, INC., a California corporation; and SCANTIBODIES LABORATORY, INC., a California corporation,

Counter-Claimants

v.

NICHOLS INSTITUTE DIAGNOSTICS, INC., a California corporation,

Counter-Defendants.

No. 02 CV 0046 B (LAB)

SCANTIBODIES CLINICAL LABORATORY, INC. AND SCANTIBODIES LABORATORY, INC.'S MEMORANDUM OF POINTS AND AUTHORITIES IN SUPPORT OF MOTION FOR SUMMARY JUDGMENT PURSUANT TO 35 U.S.C. § 102(f) FOR NONJOINDER OF CO-INVENTOR

Date: July 15, 2002
Time: 10:30 a.m.
Courtroom 2

Hon. Rudi M. Brewster

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CASES

STATUTES

MISCELLANEOUS

I. INTRODUCTION

Nichols Institute Diagnostics, Inc. ("Nichols") commenced this action for alleged infringement of U.S. Patent No. 6,030,790 (the "'790 patent"). But this case should go no further. Nichols' patent suffers from a fundamental flaw and is invalid on its face. The applicants for the '790 patent failed to identify and join all the inventors in their application to the United States Patent and Trademark Office ("PTO"). They failed to satisfy one of the fundamental conditions for obtaining a valid patent -- naming all the correct inventors. In other words, the applicants never properly "got off the starting blocks" in their efforts to obtain a valid patent from the PTO.

The issue presented in this motion is very straightforward. The Court need look only to the patent documents themselves to decide this discrete invalidity issue.

The '790 patent lists only three co-inventors on its face: Knut Adermann ("Adermann"), Dieter Hock ("Hock"), and Markus Mägerlein ("Mägerlein"). The '790 is the "national stage" of an earlier international patent application filed under the Patent Cooperation Treaty ("PCT"). The PCT provides a standard set of policies and procedures for filing a single application in multiple jurisdictions based on the same invention. 4 Donald Chisum, *Chisum on Patents,* § 14.02 [4] at n.1 (Cumm. Supp. Oct. 2001). In order for the '790 to claim the filing date of the PCT Application, it must have been the same application and the same subject matter. Indeed, as was appropriate under the PCT and the patent laws of the United States, the '790 claimed the filing date of the earlier PCT Application.

Although the '790 patent originated in the PTO as the PCT Application -- and was for the same invention -- the '790 patent does not name the same inventors. The PCT Application identified Adermann, Hock, Mägerlein ***and Wolf-Georg Forssmann*** as inventors. The '790, however, **does not include Forssmann**.

The omission of Forssmann from the U.S. application for the '790 patent renders it invalid under 35 U.S.C. § 102(f) as a matter of law. Having commenced an action based on an invalid patent, Nichols now has only two options: either promptly seek judicial correction

of the inventorship error under the procedure set forth in 35 U.S.C. § 256 or have the '790 patent declared invalid for nonjoinder of inventors. In either case, this costly lawsuit is premature and should proceed no further.

II. STATEMENT OF THE FACTS

On September 28, 1994, an application was filed with the German Patent Office, Federal Republic of Germany. *See* Ex. A attached to Declaration of M. Andrew Woodmansee in Support of Scantibodies Clinical Laboratory, Inc. and Scantibodies Laboratory, Inc.'s Motion for Summary Judgment Pursuant to 35 U.S.C. § 102(f) ("Woodmansee Decl."). That patent, DE 44 34 551 A1, identified four inventors on its face: Adermann, Hock, Mägerlein ***and Forssmann***. *Id.* According to the abstract:

> The invention relates to peptides from the human parathyroid (hPTH) sequence (1-37), containing α-helical amino acid sequence regions, where said peptides are capable of inducing antibodies when injected into animals. The invention also relates to a diagnostic agent and antibodies obtainable by vaccination of animals with the peptides in question.

(Woodmansee Decl., Ex. A at p. 3)

The German patent (DE 44 34 555 A1) formed the basis for an application filed on September 22, 1995 with the World Intellectual Property Organization and published pursuant to the PCT. The PCT Application (WO 96/10041) claimed priority on its face to the 1994 German application. (Woodmansee Decl., Ex. B at p. 12) Like its German counterpart, the PCT named the same four inventors: Adermann, Hock, Mägerlein ***and Forssmann***. (*Id.*) The abstract of the PCT Application described the same subject matter as the German application:

> The invention concerns peptides from the human parathyroid (hPTH) sequence (1-37) and containing α-helical amino acid sequence regions and/or non-structured amino acid sequence regions. The said peptides are capable of inducing antibodies when injected into animals. The invention also concerns a diagnostic agent and antibodies obtainable by vaccination of animals with the peptides in question.

(Woodmansee Decl., Ex. B at p. 12)

Based on the disclosure and filing date of the PCT Application (September 22, 1995), an application was filed with the United States Patent and Trademark Office. That application ultimately issued as the '790 patent. The abstract of the '790 patent describes its subject matter and claimed inventions as follows:

> The present invention is directed to peptides from the sequence of hPTH (1-37), which contain α-helical amino acid sequence regions and/or non-structured amino acid sequence regions, said peptides being capable of inducing antibodies when injected into animals. Furthermore, the invention is directed to a diagnostic agent and antibodies obtainable by immunizing animals using the peptides according to the invention.

(Woodmansee Decl., Ex. C at p. 30)

The application for the '790 patent was submitted to the PTO as the national stage of the PCT Application. The application claimed the benefit and filing date of the PCT Application. (*Id.*) Indeed, the named inventors on the application -- Adermann, Hock and Mägerlein -- submitted a declaration to the PTO claiming inventorship of the invention sought in the application and confirming that the specification of the invention had first been filed in the PCT Application. The inventors' declaration states, under penalty of perjury, in pertinent part:

> As a below named inventor, I hereby declare that: . . . I believe I am an original, first and joint inventor (if plural names are listed below) of **the subject matter which is claimed and for which a patent is sought on the invention** entitled: "PEPTIDES FROM THE hPTH (1-37) SEQUENCE, **the specification of which . . . was filed on September 22, 1995 as PCT International Application No. PCT/EP95/03757** . . . .

(Woodmansee Decl., Ex. D at p.62) (emphasis added).

The '790 patent identifies only three inventors, not four. (Woodmansee Decl., Ex. C at p. 30) The '790 patent identifies Adermann, Hock and Mägerlein as inventors. (*Id.*) Although he was named as an inventor in connection with the PCT Application (and the underlying German patent), Wolf-George Forssmann was never named as an inventor in connection with the application in the United States for the '790 patent.

III. ARGUMENT

A. The Naming Of The Correct Inventors Is A Condition Of Patentability

In *Pannu v. Iolab Corp.*, 155 F.3d 1344 (Fed. Cir. 1998), the Federal Circuit explained the statutory basis requiring a finding of invalidity where a patent fails to name the correct inventors. The Federal Circuit explained that Section 102 establishes the "conditions of patentability" that must be satisfied in order to obtain a valid patent. *Id.* at 1348-49. In particular, Section 102(f) provides that "[a] person shall be entitled to a patent unless -- he did not himself invent the subject matter sought to be patented." 35 U.S.C. § 102(f). "Since the word 'he' [in Section 102(f)] refers to the specific inventive entity named on the patent . . . this subsection **mandates that a patent accurately list the correct inventors of a claimed invention** . . ." *Pannu* at 1349 (emphasis added). "Accordingly, if nonjoinder of an actual inventor is proved by clear and convincing evidence, . . . **a patent is rendered invalid**. *Id.* (citations omitted) (emphasis added).

Although the failure to name the correct inventors renders a patent invalid, the Patent Act provides a procedure for correcting inventorship errors that have been established in ongoing litigation. In *Pannu*, the Federal Circuit held that the operation of section 102(f) in the context of ongoing litigation is "ameliorated" by the ability to correct inventorship errors through a judicial hearing pursuant to section 256 of the Patent Act. *Id* at 1350. "Upon such a finding of incorrect inventorship, a patentee may invoke section 256 to save the patent from invalidity." *Id.* Specifically, the Federal Circuit acknowledged: "Non-joinder may be corrected 'on notice and hearing of all the parties concerned' and upon a showing that the error occurred without any deceptive intent on the part of the unnamed inventor." 35 U.S.C. § 256. "If a patentee demonstrates that inventorship can be corrected as provided for in section 256, a district court must order correction of the patent, thus saving it from being rendered invalid." *Id.* at 1350.

B. The Undisputed Evidence Establishes That The '790 Patent Suffers From Nonjoinder Of An Inventor: Wolf-Georg Forssmann

When one compares the PCT Application with the initial application in the United States for the '790 patent, it is apparent that Wolf-Georg Forssmann should have been named as an inventor on the '790 patent. The application that issued as the '790 patent was the "national stage" in the United States of PCT Application WO 96/10041. (*See, e.g.*, Woodmansee Decl., Ex. C at p. 30; Ex. D at p. 62) The filing of the PCT Application in 1995 had the same effect as an application normally filed in the United States PTO. "An international application designating the United States shall have the effect, from its international filing date under article 11 of the treaty, of a national application for patent regularly filed in the Patent and Trademark Office except as otherwise provided in section 102(e) of this title." 35 U.S.C. § 363; *see also Chisum* § 14.02 [4] (noting that, to commence the "national phase" in the U.S. based on a prior PCT Application, the applicant must pay a filing fee, file a copy of the international application and an English translation).

"The general purpose of the PCT is to provide a single set of standards and procedures for the filing of patent applications on the same invention in any of the over ninety PCT member countries." *Chisum* § 14.02 [4]n.1 (quoting Department of Commerce, Patent and Trademark Office, Revision of Patent Cooperation Treaty Application Procedure, 63 Fed. Reg. 66040, 66041 (Dec. 1, 1998)) (emphasis added). Because the PCT process is intended to permit multi-jurisdictional patenting of the same invention, the inventorship of the national stage application should follow the inventorship designation in the originating country. *Chou v Univ.of Chicago*, 254 F.3d 1347, 1360 (Fed. Cir. 2001) (noting that inventorship on PCT and foreign national stage applications "normally follows the inventorship designation in the originating country").

Although '790 was the same application and the same invention as the PCT and the underlying German patent, Forrsmann was not named as an inventor on the '790 patent. (Woodmansee Decl., Ex. C at p. 30; Ex. D at pp. 62-63) Only Adermann, Hock and

Mägerlein were named as inventors on the U.S. application. (Woodmansee Decl., Ex. D at pp. 62-63) Only Adermann, Hock and Mägerlein signed declarations with inventor's oaths in connection with the U.S. application. (*Id.*) Although Forssmann was listed as an inventor on the PCT Application (and on the underlying German patent), he was not named in connection with the U.S. application and he did not sign a declaration and inventorship oath. Given the legal relationship between the PCT Application and the application for the '790 patent -- the same application for the same invention -- Forssmann should have been an inventor of the subject matter disclosed and claimed in the '790 patent. *See, e.g., Chou* at 1360.[1]

It is clear that Forssmann should have been named as an inventor on the application that issued as the '790 patent. He was not. The application for the '790 patent therefore failed to meet one of the most basic requirements for patentability -- naming all the correct inventors. The '790 patent is invalid and unenforceable unless Nichols seeks to correct the omission in this Court pursuant to section 256. *Pannu* at 1349.

C. Nichols Must Either Immediately Move To Correct The Inventorship Error In Court Or Have The Patent Declared Invalid

Because correct inventorship is a condition of patentability, a patentee cannot enforce a patent until it has taken the corrective steps required by statute. *Id.* at 1349 (holding that patentee "must claim entitlement to relief under the statute" or have its patent declared invalid); *see also Merry Mfg. Co. v. Burns Tool Co.*, 335 F. 2d 239, 242 (5th Cir. 1964) ("The patent is unenforceable until corrective steps are taken" and if correction cannot be made "the

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[1] Nichols may attempt some explanation for its failure to follow the normal rule that inventorship of national stage applications follows the inventorship designation in the originating country. Scantibodies submits that there is no reasonable explanation for applicants' failure to name Forssmann as an inventor of the '790 patent in light of the identical subject matter

(Footnote continues on following page.)

unenforceability ripens into invalidity"); *Mas-Hamilton Group v. LaGard, Inc.*, 21 F. Supp.2d 700, 711 (E.D.Ky. 1997) ("Such a patent with inventorship defects is unenforceable until corrective steps are taken.").

Although section 256 provides an opportunity to correct inventorship errors, Nichols cannot avoid summary judgment by asserting that the patent *might* still be corrected at the PTO. In *Pannu*, the Federal Circuit held that the mere possibility of correction under section 256 does not allow a patentee to escape invalidation of a patent due to misjoinder. In order to avoid a finding of invalidity by this Court, Nichols must immediately commence an action for judicial correction upon notice and hearing of all concerned parties.

> [A] patent with improper inventorship does not avoid invalidation simply because it might be corrected under section 256. Rather, the patentee *must claim entitlement to relief under the statute and the court must give the patentee an opportunity to correct the inventorship*. If the inventorship is successfully corrected, section 102(f) will not render the patent invalid. On the other hand, if the patentee does not claim relief under the statute and a party asserting invalidity proves incorrect inventorship, the court should hold the patent invalid for failure to comply with section 102(f).

Pannu at 1350-51 (emphasis added). The Federal Circuit held in the context of ongoing litigation "[n]onjoinder may be corrected 'on notice and hearing of all parties concerned.'" *Id.* at 1350. Now that incorrect inventorship has been raised in this action, that hearing can only take place in this Court.

In short, Nichols is before this Court with a clearly invalid and/or unenforceable patent. Nichols should not be allowed to proceed with an action for infringement of the '790 patent under that circumstance. Nichols has two choices: either it must immediately commence an action for

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(Footnote continued from previous page)

described in the related applications, and looks forward to addressing whatever excuses Nichols may proffer.

judicial correction of inventorship under section 256, or else face this Court's ruling that the '790 patent is invalid for incorrect inventorship. *See id.*

A similar situation was addressed by the Massachusetts district court in *PerSeptive Biosystems v. Pharmacia Biotech*, 12 F. Supp.2d 69 (D.Mass. 1998). In *PerSeptive Biosystems*, the district court granted defendant's cross-motion for summary judgment that plaintiff's patents were invalid for nonjoinder of the true inventors. *Id.* at 75. The court ordered the plaintiff to move to correct inventorship pursuant to section 256 within ten days of the summary judgment order, or the action would be dismissed. *Id.* at 87. Plaintiff subsequently moved for judicial correction in the district court.

Scantibodies respectfully requests that this Court follow a similar procedure in this case. In light of the undisputed evidence and the Federal Circuit's ruling in *Pannu*, Scantibodies requests that the Court require Nichols to commence within ten days an action for judicial correction of inventorship pursuant to section 256 on notice and hearing of all interested parties. If Nichols does not promptly do so, Scantibodies requests that the Court enter summary judgment of the invalidity of the '790 patent under section 102(f). *Pannu* at 1350-51.

D. If Nichols Seeks Judicial Correction Of Inventorship, The Infringement Action Should Be Stayed Pending Hearing On Inventorship Issues

Assuming Nichols does seek judicial correction pursuant to section 256, Scantibodies requests that the action against it for allegedly infringing the '790 patent be stayed pending full discovery and hearing of the inventorship issues. A costly action for infringement of a patent that is invalid for nonjoinder of inventors is "premature" while the inventorship remains uncorrected. *See Burroughs Wellcome Co. v. Barr Lab.*, 40 F.3d 1223, 1227 (Fed. Cir. 1994) ("If Barr and Novopharm are correct, then [the omitted inventors] should have been named as joint inventors and the resolution of Burroughs Wellcome's infringement suit

is premature."). Scantibodies should not be forced to spend any additional sums on a premature infringement suit that should not even have been filed unless and until the nonjoinder of true inventors had been corrected.

IV. CONCLUSION

This motion presents a simple issue as to which there can be no genuine disputes. The '790 patent is invalid due to nonjoinder of Forssmann. Nichols must now seek judicial correction of inventorship on notice and hearing of all concerned parties or have its patent declared invalid pursuant to section 102(f). In either case, Nichols should not be permitted to force Scantibodies to expend further resources on an infringement action that is, as a matter of law, premature.

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